EVALUATION OF CO-EXISTENCE OF *Helicobacter pylori* AND *Candida* IN THE ORAL CAVITY OF DYSPEPTIC PATIENTS

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CERTIFICATE

This is to certify that this dissertation titled "**EVALUATION OF CO-EXISTENCE OF *Helicobacter pylori* AND *Candida* IN THE ORAL CAVITY OF DYSPEPTIC PATIENTS**" is a bonafide dissertation performed by **PREM KARTHICK. B** under our guidance during the postgraduate period 2009-2012.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY** in **ORAL PATHOLOGY AND MICROBIOLOGY, BRANCH VI**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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CONTENTS

Introduction

Oral cavity with its unique anatomical location and its role in physiological processes such as digestion and speech, acts as a window to the body. Oral cavity involvement precedes the appearance of other signs and symptoms of many systemic diseases. Signs and symptoms of many systemic diseases such as haematological, gastro-intestinal, endocrine, dermatological and nutritional deficiency associated lesions are first observed in the oral cavity.[1]

The oral cavity is inhabited by various Gram positive and Gram negative micro-organisms. The colonization of oral cavity by microbial flora are influenced by factors such as temperature, pH, nutrient supply and hormonal balance.[2]

Saliva is the product of salivary glands. It contains water mixed with micro-organisms, microbial products, food debris, upper airway secretions, minerals, buffers and enzymes. Saliva has been used as an investigational aid in the oral diagnosis of various systemic diseases and infections.[3]

The presence of spiral or curved bacilli in close association with human gastric mucosa was first described as early as 1896.[4] Helicobacter pylori was demonstrated by Warren JR and Marshal B in 1983 as a gram negative micro-aerophilic spiral shaped bacterium measuring 2.5 to 5 μm in length recognized as an etiological agent of chronic active gastritis and peptic ulcer disease and also has been associated with gastric cancer.[5, 6]

The natural niche of *Helicobacter pylori* is the gastric mucosa, however, investigators have detected *Helicobacter pylori* DNA by PCR amplification in

extragastric sites such as oral cavity and oesophagus.[4,7-17] Within the oral cavity, *Helicobacter pylori* is commonly detected in the dental plaque[18-23]especially around the molar tooth[18] due to decreased oxygen exposure which favours the growth of this micro-aerophilic bacterium. *Helicobacter pylori* has been detected from the tonsils, adenoids[24] and finger nails.[12]

Helicobacter pylori detection by polymerase chain reaction has targeted various genes such as cytotoxin vacuolating gene vacA s1s2 [25], 16S rRNA gene[7,11,19,23], Urease A gene [9,10,15], Urease AB gene.[25]

Candidiasis is caused by an ubiquitous fungi, *Candida.* It is prevalent in 40 - 60% of healthy human oral cavities. Deficiency of the host defences create a favourable environment for the fungi to multiply and cause multiple pathologies.[26] Candidiasis has been associated with debilitating immune disease such as HIV, infectious disease such as leprosy,[26] metabolic diseases such as diabetes mellitus[27,28] and also betel quid chewing habit[26]. *Helicobacter pylori* and candida have been detected in close association in the oral cavity and the upper gastrointestinal tract.[25,29,30] Non culturable bacterium like bodies (BLBs) of *Helicobacter pylori* have been detected within the vacuoles of the yeast, *candida.* This association was detected in the oral cavity by polymerase chain reaction[25]. Similar association has been detected in the upper gastrointestinal tract of patients with peptic ulcer[30]. This symbiotic relationship between *candida* and *Helicobacter pylori* might play a major role in the persistence of

Helicobacter pylori within the oral cavity and the stomach and thereby could cause recurrence of *Helicobacter pylori* infection treated by antimicrobials and proton pump inhibitors.

In this study, we intend to assess the presence of *Helicobacter pylori* bacterium and *candida* in the oral cavity of symptomatic gastritis patients with and without *Helicobacter pylori* in the gastric mucosa and also to evaluate the relationship between them in the oral cavity and to evaluate whether oral cavity is the reservoir of *Helicobacter pylori.*

Aims & Objectives

Null Hypothesis:

Helicobacter pylori and *Candida* do not co-exist in the oral cavity of patients with recurrent dyspepsia.

Alternate Hypothesis:

Helicobacter pylori and *Candida* exist together in the oral cavity of patients with dyspepsia.

Aim:

To standardize the technique of detection of *Helicobacter pylori* genome by polymerase chain reaction and to evaluate the presence of *Helicobacter pylori* and *Candida* in the oral cavity in the following groups.

Group I- Symptomatic gastritis patients with the presence of *Helicobacter pylori* in their gastric mucosa.

Group II – Symptomatic gastritis patients without *Helicobacter pylori* in their gastric mucosa.

Group III –Asymptomatic healthy controls without any systemic disease and medication.

Objectives:

To evaluate the presence of

- *Helicobacter pylori* in the oral cavity of Dyspeptic patients by Polymerase chain reaction.
- *Candida* species in oral cavity of dyspeptic patients by candidal culture.
- The co-existence of *Helicobacter pylori* and *Candida* of oral cavity in recurrence of Chronic Gastritis.

Gastritis is an inflammation of the gastric mucosa with central abdominal pain, belching, bloating and retrosternal pain. Gastritis is most frequently caused by *Helicobacter pylori* and Non steroidal anti-inflammatory drugs (NSAIDs).

Materials & Methods

Definition of Dyspepsia:

Dyspepsia refers to a condition of impaired digestion characterized by abdominal pain, abdominal fullness and belching and heart burn. Dyspepsia occurs most commonly due to gastritis and gastroesophageal reflex disease.

Characterization of Symptomatic Gastritis patients and control:

Gastric Helicobacter pylori Positive (Group I): Presence of curved spiral Gram negative bacteria in the gastric mucosa (*Helicobacter pylori*) collected by gastric endoscopy.

Gastric Helicobacter pylori Negative (Group II): Absence of curved spiral Gram negative bacteria in the gastric mucosa (*Helicobacter pylori*) collected by gastric endoscopy.

Controls (Group III): Asymptomatic, apparently normal controls.

Inclusion criteria for Symptomatic gastritis patients:

- Patients with chief complains of abdominal pain, belching, bloating and retrosternal pain who were going to undergo Endoscopy.

Exclusion Criteria for Symptomatic gastritis patients:

- Patients who suffer from acute dyspepsia with other related co-morbidities and patients on antifungal and proton pump inhibitors medications within the previous 4 weeks.

Study Subjects:

Group I – 30 patients with symptomatic gastritis and presence of *Helicobacter pylori* in gastric mucosa.

Group II – 30 patients with symptomatic gastritis without *Helicobacter pylori* in gastric mucosa.

Group III – 10 Asymptomatic healthy controls without any systemic disease and medication.

SAMPLE:

- Saliva specimens were collected from patients in sterile containers after taking a brief medical history before Endoscopic procedure between 9.30am to 12.30pm.

PROCEDURE:

STUDY SITE, STUDY SUBJECTS AND SAMPLE COLLECTION:

- The patients attending the endoscopy department of the Billroth Hospital, Chennai with a chief complains of abdominal pain, belching, retro-sternal pain and bloating were interviewed and short medical history was collected.
- The subjects were informed about the study and an informed consent was given by the subject.
- The subjects were asked to sit in an upright position and a 50 ml sterile plastic saliva collection container was given to them.
- The subjects were asked to spit the saliva every 1 minute into the container for 10 minutes
- The Saliva samples were kept in an ice box and transported to the department of Oral Pathology, Ragas Dental College, Chennai and stored in the deep freezer at -70^{o}C.

ARMAMENTARIUM:

1. For patient examination and sample collection
    - Gloves and mouth masks
    - Sterile mouth mirror and straight probe
    - 50 ml sample collection containers
2. For DNA extraction
    - Microcentrifuge tubes
    - Positive displacement pipettes
    - DNA extraction kit **[Figure 1]**
    - Thermostat
    - Cooling centrifuge **[Figure 3]**
3. For DNA amplification
    - Thermal cycler **[Figure 6]**
    - Urease and 16 S rDNA gene specific primer
    - Housekeeping gene primers
    - PCR master mix
    - Positive control.
4. Gel electrophoresis
    - Agarose
    - TAE (Tris Acetate EDTA)
    - Ethidium bromide
    - Horizontal electrophoresis unit **[Figure 7]**
    - Microwave
    - Gel Dock system **[Figure 8]**

IDENTIFICATION OF *Helicobacter pylori* USING POLYMERASE CHAIN REACTION:

PROCEDURES IN *Helicobacter pylori* IDENTIFICATION BY POLYMERASE CHAIN REACTION:

- Extraction of DNA
- Amplification of target by Polymerase Chain Reaction
- Gel Electrophoresis

EXTRACTION OF *Helicobacter pylori* DNA:

Saliva samples were taken from the deep freezer and kept for thawing till they reached the room temperature.

- 1500µl of saliva was taken in a 1.5ml micro centrifuge tube. **[Figure 5]**
- The tube was Centrifuged for 5 minutes at 13000rpm.1200µl of the supernatant was discarded from it.
- 900µl of Real Biotech Corporation (RBC) lysis buffer was added to it and mixed by inversion method.
- The tube was incubated for 5 minutes at room temperature.
- It was then centrifuged at 1300 rpm for 5 minutes and the supernatant was discarded.
- 100 µl RBC lysis buffer was added to resuspend the cell pellet.
- 200µl GB buffer was then added to the tube and mixed by vortex.
- The mixture was incubated at room temperature for 10 minutes until the sample lysate became clear. During incubation, the tube was inverted every 3 minutes.

- The required quantity of elution buffer (200µl / sample) was preheated in a 70° C water bath (for DNA elution).
- 200µl of ethanol (96-100%) was added to the sample lysate and was mixed immediately in the vortex machine **[Figure 2]** for 10 seconds.
- GD (a tube with a sieve) column was placed in 2ml collecting tube**[Figure 4]**.
- Total mixture (including any precipitate) from the previous step was added to the GD column.
- The cap of the column was closed and centrifuged at 13000rpm.
- 200µl of GB buffer was added to it and centrifuged at 6000rpm for 5 minutes.
- 400 µl of W1 buffer was added into the GD column and centrifuged at 13000 rpm for 30 seconds.
- The flow-through was discarded and GD column was placed back in the 2ml collecting tube.
- 600 µl of wash buffer was added into the GD column.
- It was then centrifuged at 13000 rpm for 30 seconds, the flow-through was then discarded and the GD column was placed back in the 2ml collecting tube.
- It was then centrifuged at 13,000 rpm for 5 minutes to dry column matrix.
- Dried GD column was then transferred into clean 1.5 ml micro-centrifuge tube.
- 100 µl of preheated elution buffer was added into the centre of column matrix.
- It was then allowed to stand at room temperature for 5 minutes until elution buffer is absorbed by the matrix and centrifuged at 13000 rpm for 30 seconds to elute purified DNA.
- The extracted DNA was stored at - 4^{o}C till it was further used.

DNA AMPLIFICATION USING PCR:

- The primers PC04 and GH20 were selected to amplify the β chain of haemoglobin to validate the PCR amplification of genomic DNA.
- PC04 and GH20 primers yielded a product of 268 base pairs.
- HP001/HP002 primers were used to amplify the 16S rRNA gene of *Helicobacter pylori.*
- HP001/HP002 primers did not yield any product corresponding to 534 base pairs.
- The primer sequences were as follows:

β globin primer

PC04 : CAA CTT CAT CCA CGT TCA CC

GH20 : GAA GAG CCA AGG ACA GGT AC

16S rRNA primers:

HP001: 5'-TAA GAG ATC AGC CTA TGT CC-3'

HP002: 5'– TCC CAC GCT TTA AGC GCA AT- 3'

- The primers, DNA extracted from the sample, master mix, positive control and milli Q water were taken out from the deep freezer and kept for thawing.
- The laminar flow was cleaned with acetone and UV light was switched on for 30 minutes.
- 50 µl reaction mixtures were prepared in the laminar flow unit using positive displacement pipettes as follows:

	Positive control	Sample	Negative control
Master mix	25 µl	25 µl	25 µl
MY 09 primer	1 µl	1 µl	1 µl
MY 11 primer	1 µl	1 µl	1 µl
PC 04 primer	1 µl	1 µl	1 µl
GH 20 primer	1 µl	1 µl	1 µl
DNA	6 µl of H. pylori DNA	6 µl sample DNA	6 µl of millipore water
Millipore water	15 µl	15 µl	15 µl

Positive and negative controls were included in each PCR amplification cycle. The final 50µl reaction mixture was amplified in a thermal cycler **[Figure 6]**. The amplification programme was as follows:

Number of cycles	Temperature	Time
1	Initial denaturation 95 ^{o}C	5 minutes
40	Denaturation 94 ^{o}C Annealing 56 ^{o}C Synthesis 72 ^{o}C	30 seconds 30 seconds 1 minute
1	Final extension 72 ^{o}C	5minutes

Positive control showed a beta globin band corresponding to 268 base pairs but Helicobacter pylori band corresponding to 534 base pairs of DNA ladder was absent. The negative control did not have any band. All Samples showed a beta globin band whereas the Helicobacter pylori band was not present.

REASON FOR OPTING REAL TIME PCR:

PCR was done using the genomic DNA isolated from the saliva to detect the presence of the 16S rDNA gene in H. pylori. On repeating the procedure for amplification of 16S r DNA gene, we were not able to convincingly amplify the DNA sequence. This was revealed in Gel electrophoresis as there was no band corresponding to amplified product at 534 base pairs. We counterchecked the technique but we found that β globulin was amplified in all the cycles. So, real time PCR was done to identify the presence of helicobacter pylori targeting the urease A gene.

The real time PCR for the detection of helicobacter pylori showed the presence of helicobacter pylori. The sample was considered positive, if the fluorescence levels increased above threshold level.

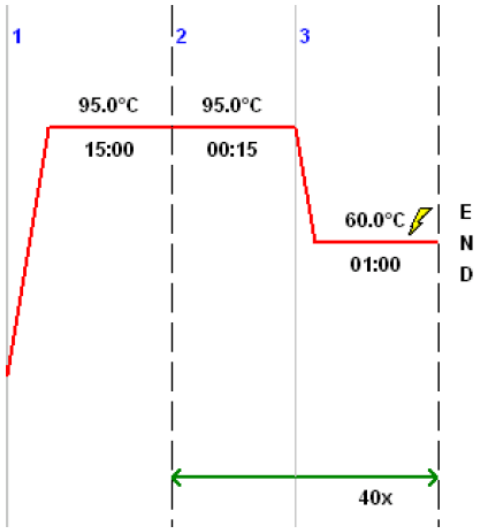
HELICOBACTER PYLORI IDENTIFICATION BY REAL TIME PCR:

Real time PCR also called quantitative real time polymerase chain reaction(Q-PCR/qPCR/qrt-PCR) or kinetic polymerase chain reaction (KPCR) was used. For the amplication procedure, 25µl of PCR assay was prepared by adding master mix from Qiagen and specific primer and probe for Urease A gene into the DNA extracted from the samples (template DNA). Detection was done using sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter (FAM) which permits detection only after hybridization of the probe with its complementary DNA target.

Preparation for 25 µl of PCR assay reaction

Master mix (Qiagen)	-	12.5µl
Primer and probe for Urease gene	-	4 µl
Template DNA	-	5 µl
Millipore Water	-	3.5µl

The 25µl of assay prepared from the extracted DNA of all samples were transferred into Eppendorf PCR cuvettes. These cuvettes along with the content were centrifuged in minispin for 5 seconds and then transferred into Eppendorf master cycler to run the Real Time PCR. The PCR was programmed to run for 40 cycles with a standard cycle consisted of annealing at 95^{o}C for 15 seconds followed by extension at 60^{o}C for 60 seconds



Primers used in Real Time PCR:

Forward Sequence: GGC AAA AAA GCC GTT AGC GTG AA

Reverse Sequence: TGT CCC GCT CGC AAT GTC TAA G

Probe Sequence: ATGTTGGCGACAGACCGGTTCAAATCGGCTCACAC

GEL ELECTROPHORESIS:

2% agarose gel was prepared using 1xTAE buffer and agarose powder. 1.20 grams of agarose powder was weighed and transfered into a clean conical flask, washed with deionised water. To this 60ml of 1xTAE buffer was added and it was heated in a microwave oven for one and a half minute. The conical flask was taken out after every 30 seconds and swirled to mix. It was then allowed to cool.

Meanwhile, the gel electrophoresis tank **(Figure 7)**, boat and comb were washed using deionised water. The ends of the gel boat were sealed using adhesive tape. 15µl Ethidium bromide was added to the agarose solution, it was swirled well and poured into the gel boat having the gel comb. The agarose was allowed to cool and gel. The gel comb was then removed slowly to leave behind wells in the gel. The adhesive tape was removed and the boat was placed in the tank. 1xTAE was poured into the tank so as to immerse the gel completely.

2µl of loading dye was mixed with 8µl of 100 base pairs DNA ladder and was loaded into the well in the gel. 8µl of sample, positive control and negative control were mixed with 2µl of loading dye separately and loaded into the wells. The gel tank was connected to the electrophoresis unit which delivered 100v electric current for 45 minutes. The electrophoresis unit was disconnected and the gel was taken out and placed on an UV illuminator and visualised using Gel Dock system **(Figure 8)**. The images of the gel was captured and stored.

IDENTIFICATION OF CANDIDA ALBICANS:

COMPOSITION AND PREPARATION OF SABOURAUD DEXTROSE AGAR (HIMEDIA, India) Figure 9:

Mycological peptone - 10gm/L

Dextrose - 40 gm/L

Agar - 15 gm/L

Distilled Water - 1000ml

- 65 gm of SDA agar was suspended into 1000 ml of distilled water and heated to boiling using Bunsen burner in a laminar flow to dissolve the medium completely.
- The medium was sterilized by autoclaving at 15lbs pressure 121^{o}C for 15 minutes and allowed to cool.
- The medium was then poured into sterile petri dishes **(Figure 10)** and kept in the laminar flow until it solidifies.
- The saliva was taken in a 0.001 µl loop, inoculated by streaking in the medium and placed in an incubator at 37^{o} C for 48 hours.
- After 48 hours of incubation at 37^{o} C, growth of any candidal colonies was recorded as a positive growth and the subject was considered as a candidal carrier. **(Figure 18, 19)**

Statistical Analysis

STATISTICAL ANALYSIS:

Data entry and database management were doing using Microsoft Office Excel 2003. Statistical analyses were done using Statistical Package for Social Sciences (SPSS) version 11.0.1. Chi-square test, t- test/ Mann – Whitney U test and ANOVA/ Kruskal-Wallis Non-parametric test was utilized wherever appropriate. For normally distributed data, linear association between any two variables was done by Pearson Correlation. In the case of non-normal data, non-parametric measure of correlation, Spearman Correlation Coefficient was presented. Odds ratio and 95% confidence interval also presented. p-value <= 0.05 were considered as statistical significance.

Review of Literature

PEPTIC ULCER- PREVALENCE IN INDIA

Frank Tovey [31] in 1979 tried to estimate the prevalence of peptic ulcer in the Indian sub-continent and so they reviewed literature from 1877 to 1978. They found that peptic ulcer occurred more commonly in Karnataka, Kerala, Tamilnadu, Andhra Pradesh, southern part of Maharashtra, Orissa, West Bengal, plains of Assam, Meghalaya, parts of Kashmir and Bangladesh. These distributions are areas of high humidity, where rice is the staple food. They also found that duodenal ulcer occurred more commonly than gastric ulcer and men were affected more commonly than women. The peak age of prevalence was between 30 – 40 years of life. They found that duodenal ulcer occurred predominantly in the lower income group. South Indians living in other countries have the same predisposition for duodenal ulcer as the people in the sub-continent suggesting that climatic variations do not contribute for the susceptibility. They have also postulated that gastric acid secretion, pepsin secretion, intake of spices, malnutrition, infrequent meals, smoking, beverages consumption, hookworm infestations, pantothenic acid deficiency, refined or unrefined carbohydrate food, masticatory and non-masticatory diet could contribute as etiological factors for duodenal ulceration. They also compared the unrefined wheat and rice group and found that both do not have any protective mechanism but the supplements taken along with it like cabbage, lady's finger, pulses like horsegram, soyabeans and whole cream milk could protect the gastrointestinal tract from ulcers.

Khuroo MS, Mahajan R, Zargar SA[32]**et al** in 1989 conducted a study at Srinagar, Kashmir, India, where 2763 adults aged above 15 years were interviewed with predefined questionnaires. The sample comprised of patients with symptoms of gastric ulcer disease and patients without any symptoms of

gastric ulcer disease. All the interviewed subjects were asked to report to the Department of Gastroenterology at Sher-i-Kashmir Institute of Medical Sciences for endoscopy. 370 patients responded, of which 193 subjects were with symptoms of gastric ulcer disease and 177 subjects were without any symptoms of gastric ulcer disease. The patients were sent to the department for endoscopy. They found that in these patients duodenal ulcer was more prevalent than gastric ulcer and the ratio was 17.1:1. The duodenal ulcer was twice more common in men than in women and gastric ulcer was four times more common in men than in women. The prevalence of peptic ulcer increased in 4thdecade and peaked at the 5th decade of life. They considered that the clinical behaviour of peptic ulcer in India is different from that of the West, as peptic ulcer is less likely to bleed or perforate and more likely to cause stenosis and gastric outlet obstruction.

PEPTIC ULCER AND *HELICOBACTER PYLORI*

Virendra Singh, Bandana Trikha, Chander Kanwal Nain[33]et al in 2002 conducted a study at Chandigarh, India, to evaluate the prevalence of peptic ulcer disease. 2649 individuals were screened and 254 individuals attended the outpatient service and 147 underwent endoscopy. Among the 147 individuals who underwent endoscopy 80 were symptomatic and 67 were asymptomatic for gastritis. Biopsy specimens were collected from antrum, pylorus and corpus for rapid urease test and histological examination. Apart from biopsy specimens, serum samples were collected for evaluation of anti- *Helicobacter pylori* immunoglobulin G. They found that 13 of the 147 subjects had peptic ulcer and *Helicobacter pylori* was found in 11 of those 13 subjects in the gastric mucosa. They also found that duodenal to gastric ulcer ratio was 12:1 and most of the patients were in their 4th and 5th decade of life. In histological examination, most

common change noted was chronic superficial gastritis. They found that peptic ulcer disease showed an equal sex predilection and socio-economic status did not have an impact on the prevalence of peptic ulcer disease. They found that 11/80 (13.7%) dyspeptic patients had peptic ulcer disease. They found that *Helicobacter pylori* infection was seen in 38/67 (56.7%) of asymptomatic, 49/80(61.3%) of symptomatic and 87/147 (59.2%) in the overall population. They also found that none of the asymptomatic patients with *Helicobacter pylori* infection had an active peptic ulcer disease.

Kalyanakrishnan Ramakrishnan and Robert C Salinas[34] in 2007 reviewed articles to establish the cause of peptic ulcer disease and found that peptic ulcer disease (PUD) occurred due to gastric mucosal damage secondary to pepsin and gastric acid secretion. This peptic ulcer disease occurred more commonly in the stomach and proximal duodenum. Nearly 70% patients with PUD occurred between 25 -64 years of age. The symptoms of peptic ulcer disease included episodic gnawing or burning epi-gastric pain, loss of appetite, intolerance to fatty foods and heart burn. The most common cause of PUD has been suggested for *Helicobacter pylori* infection, drugs such as non steroidal anti inflammatory drugs (NSAIDs), steroids, bisphosphonates, potassium chloride, chemotherapeutic agents and Zollinger-Ellison syndrome. Stress could also be a causative factor for PUD. They also suggested that smoking increases risk of ulcer recurrence and slows the healing process. They convey that the *Helicobacter pylori* bacterial adherence to the gastric mucosa, the presence of outer inflammatory protein and a cytotoxin- associated gene island in the bacterial chromosome increases the virulence and ulcerogenic potential of the bacterium. They also found that patients with *Helicobacter pylori* infection have increased gastrin level and

decreased gastric mucus production, duodenal mucosal bicarbonate secretion, all of which could favour ulcer formation. They also found that eradication of *Helicobacter pylori* reduces the incidence of ulcer recurrence from 67% to 6% in patients with duodenal ulcers and from 59% to 4% in patients with gastric ulcers. They felt that esophago-gastro-duodenoscopy(EGD) is more sensitive and specific for peptic ulcer disease. They also suggested that if *Helicobacter pylori* infection was found in the gastric mucosa of the peptic ulcer disease patients it should be immediately eradicated and anti-secretory drugs such as proton- pump inhibitors should be advocated for four weeks.

Helicobacter pylori have an enzyme urease which has the capability to split urea into ammonia and carbon di oxide. Various studies have incorporated radio-isotope labelled carbon for the detection of *Helicobacter pylori* in the infected subjects. **Tanvir Ahmad, Rakhshanda Bilal and Azra Khanum**[35] conducted a study in the suburbs of Islamabad, Pakistan to determine the prevalence of *Helicobacter pylori* infection in normal school going children. 400 school going healthy children between 3 and 16 years of age were recruited for the study. Urease breath test was conducted and nearly 72.3% of apparently healthy children were found to harbour *Helicobacter pylori*. The prevalence rate increased from 3 to 10 years of ageand was followed by a decrease. This lower prevalence in the children more than 11 years of age could be attributed to increased antibody production with age and also to better sanitary habits of these children than younger children.

ISOLATION OF *HELICOBACTER PYLORI*

FarhatRizviand Abdul Hannan [36] in 2000 conducted a study in Pakistan to evaluate the prevalence of *Helicobacter pylori* infection in gastric biopsy specimens to determine suitable transport and culture medium for *Helicobacter pylori*. 100 patients complaining of upper gastro-intestinal tract symptoms were recruited for the study and two gastric antral biopsies were collected. One of the biopsy was transported in semi solid motility medium (SSMM) and other in thioglycollate broth. All the specimens were crushed in sterile tissue grinder and cultured in three different media i.e. Brain heart infusion Agar plus 7% Horse Red Blood Cells (HRBC) plus SR69(antibiotic supplement), Chocolate Agar and Columbia Agar plus 7% HRBC plus growth supplement. They found that the growth obtained was 44% and 42% in biopsies transported in SSMM and thioglycollate broth respectively. Thus, there was no significant difference between the two transport media. Brain heart infusion agar plus 7% HRBC plus SR69(antibiotic supplement) was found to be the best of the 3 culture media for the isolation of *Helicobacter pylori* with a growth rate of 45%. They conclude that proper transport and selection of culture media is required for optimal isolation from gastric biopsies.

Suhaila N, Hussin S and Rahman MM[37] in 2010 conducted a study in Kuala Lumpur, Malaysia to diagnose *Helicobacter pylori* in the gastric mucosa and to compare the different methods for their efficacy, sensitivity and specificity in diagnosing *Helicobacter pylori*. 157 dyspeptic patients who underwent oesophago-gastro-duodenoscopybetween June 2007 to September 2008were recruited for the study. They performed biopsy for in-house rapid urease test (iRUT), culture and histopathology. Stool samples were collected for

Helicobacter pylori antigen detection by immunochromatography. They found that efficacy of detection of *Helicobacter pylori* by in-house Rapid urease test, culture, histopathology and Immuno Card STAT were 31.8, 13.9, 30.3 and 32.8% respectively. They found that iRUT had high sensitivity and specificity i.e. 91.5% and 93.6% and positive predictive value and negative predictive value of 86% and 96.3%, respectively. They also found the sensitivity, specificity, positive predictive value and negative predictive value in Immuno Card STAT immunochromatography were 100%,79.3%, 50% and 100%, respectively. They considered that positive culture or histopathology as the gold standard for diagnosis. They concluded that iRUTwas a good non-invasive screening tool and Immuno Card STAT rapid test can be used as an alternative non-invasive screening tool for paediatric patients.

ROUTE OF TRANSMISSION OF *HELICOBACTER PYLORI* INFECTION

Guy D Eslick[38] in 2000 in their review of *Helicobacter pylori* comprising of published literature from January 1983 upto August 2000 stated that *Helicobacter pylori* could be transmitted sexually apart from the common oral-oral and faecal-oral routes. Even though, vagina could possibly contribute for sexual transmission in low rates, it could contribute more importantly in female sex workers ethnic minorities (Australian Aborignies, African-American, Hispanics etc) and couples with increased sexual activities. Guy D Eslick also reviewed a case of vertical transmission from infected mother to the child. The vagina with the stratified squamous epithelium similar to the oral cavity could inhabit the organisms. From his review, he summarized that routes of sexual transmission include oro-anal, oro-genital, oro-oral transmission and fomites also. He

concluded that future studies should plan to determine the exact mechanism of *Helicobacter pylori* transmission.

Allaker RP, Young KA, Hardie JMet al[15] in 2002 conducted a British study to determine the prevalence of *Helicobacter pylori* in the stomach, gastric juice, oral cavity and faeces of paediatric patients. 100 children who had undergone upper gastro-intestinal tract endoscopy for various gastric problems were recruited for the study. Oral and rectal swabs along with supragingival dental plaque were collected and were placed in 1 ml of Brain heart infusion (BHI) broth. During endoscopy procedure, gastric juice and antral gastric biopsies was collected. *Helicobacter pylori* was detected in antral gastric biopsies by Rapid Urease Test, culture, PCR and histology in 13 of 100 patients, 13 of 100 patients, 20 of 100 patients and 15 of 100 patients, respectively. Overall, 22 of 100 children were detected with H. pylori in their stomach and cagA gene was found in 7 of the 20 patients who were gastric biopsy PCR-positive by the urease A method. They also found that male children were 3.6 times more likely to be gastric biopsy-positive than females. The prevalence of *Helicobacter pylori* in gastric juice, dental plaque, oral swabs and rectal swabs was seen in 11%, 36%, 23% and 8%, respectively. They found that *Helicobacter pylori* was not cultured from the oral cavity or faecal samples, indicating that a viable reservoir of the organism outside the stomach is unlikely. The PCR result in contrast showed that the organism was present in 15 of 22 (68%) of the oral cavity and 3 of 12 (25%) of the faeces of children who were positive for *Helicobacter pylori* in the gastric biopsy. This indicates that the oral-oral transmission may be the predominant mode of spread of *Helicobacter pylori* in children. They also suggest that faecal-oral transmission may play a lesser role in the spread of the organism.

ORAL CAVITY –ROLE IN TRANSMISSION OF *HELICOBACTER PYLORI* INFECTION

Donald A Ferguson Jr., Chuanfu Li, Nikhil R. Patel et al[13] in 1993 conducted a study in American population to isolate *Helicobacter pylori* from the saliva of patients with upper gastrointestinal symptoms. 16 patients with upper gastrointestinal symptoms were recruited for the study. 2 to 3 ml of saliva samples were collected prior to endoscopy and two antral biopsy specimens were taken from each patient, one for histological examination and the other for microbiological culture. The selective, blood containing medium was prepared with a brain heart infusion agar base containing 10% sheep blood and with anti-microbial agents such as (VCNT) vancomycin(4μg/ml), colistin(7.5μg/ml), nystatin(1,250U/ml) and trimethoprim lactate (5μg/ml). Two plates of selective medium were innoculated with saliva samples. In one plate, the contents of 3 swabs dipped in saliva were spread without streaking, while the second plate was streaked with contents of one swab to obtain isolated colonies. Gastric biopsy specimen were inoculated and streaked on both the sheep blood agar plate and the selective differential agar Belo-Horizonte medium. Plates were incubated at 37^{o} C in a micro-aerophilic environment for 5 days. They found that *Helicobacter pylori* from saliva had grown in one of the 9 patients who were positive by culture of antral mucosa. The presence was confirmed by morphology, gram stain, oxidase, catalase, rapid urease test, electrophoresis of soluble proteins on polyacrylamide slab gels, restriction endonucleases analysis of their DNA with Hae III and Hind III endonucleases and by Southern blot hybridization with a DNA probe developed in their laboratory. They concluded that their observation of viable *Helicobacter pylori* from saliva was the first such observation.

Li C., Musich PR, Ha T, et al[14]in 1995 conducted a study in American population to investigate the prevalence of *Helicobacter pylori* in the saliva of patients infected with gastric *Helicobacter pylori* infection. 63 patients who underwent endoscopic examination were recruited and gastric biopsy was performed. Saliva samples were also collected. All biopsy specimens were cultured and histologically examined using modified Steiner (silver) stain for identification of *Helicobacter pylori*. 1 to 2 ml of saliva specimens were collected from 56 of the 63 patients before undergoing endoscopy. The patients were asked to expectorate the saliva directly into new sterile containers containing digestion buffer. They found that *Helicobacter pylori* DNA amplified by PCR was positive in all 39 cultured positive biopsy specimens and also in 7 biopsy specimens which were negative by culture but positive by histology. They found that *Helicobacter pylori* DNA by PCR was negative in all 17 biopsy specimens which were negative by both histology and culture. They also found that oral colonization of the bacterium, *Helicobacter pylori* could precede the gastric infection and concluded that the oral cavity harbours *Helicobacter pylori* and may be the source of infection and transmission.

Dowsett SA., Archila L., Segreto VAet al[12]in 1999 conducted a study to determine the presence of *Helicobacter pylori* infection in a family. 242 subjects in nuclear family units comprising of parents and children were recruited and a complete history was elicited. Immunoglobulin G antibody serology test were performed and samples from the periodontal pockets and nail beds were collected to perform nested PCR. They found that 141 (58%) of 242 subjects were sero-positive for *Helicobacter pylori* and 209 (87%) of 242 subjects had at least one oral site positive for *Helicobacter pylori*. They also found that there was a

positive correlation between the serostatuses of mother and child. They found that *Helicobacter pylori* were present beneath the nail of the index finger of each subject's dominant hand. 136 (58%) of 233 subjects had a positive fingernail and they also found a positive relationship between fingernail and tongue positivity. They suggested that close contact, crowding, poor sanitation, lack of hot water, lack of an external water supply and coffee drinking could be few risk factors for the high infection rates in developing countries. They concluded that oral carriage of *Helicobacter pylori* may play a role in the transmission of infection and that the hand may be instrumental in the transmission of the infection.

B Marshall, A J Howat and P A Wright[17] in 1999 conducted a study in English population of 81 subjects attending for upper gastro-intestinal endoscopy. Antral biopsy specimens, serum and oral fluids were collected for detection of *Helicobacter pylori* infection. The presence of *Helicobacter pylori* infection was determined by culture, histology and urease detection. Anti *Helicobacter pylori* specific IgG was detected in serum and oral fluid by enzyme linked immune sorbent assay. 34 of 81 subjects were *Helicobacter pylori* positive by at least one of the gold standard tests. They found ELISA to have 94% sensitivity and 85% specificity in detecting *Helicobacter pylori* in oral fluids. The serum ELISA had a sensitivity and specificity of 91%. Therefore they found that the oral fluid ELISA could be a reliable, non-invasive method for the diagnosis of *Helicobacter pylori* infection.

Song Q, Lange T, Spahr Aet al[18] in 2000 conducted a German study to determine the prevalence of *Helicobacter pylori* in the dental plaque samples from different locations of the oral cavity. 42 patients who underwent gastro-intestinal

endoscopy were recruited and 13C-urea breath test was performed. Dental plaque and saliva samples were collected. By 13C-urea breath test, they found that 11 of 42 patients had an active *Helicobacter pylori* infection and 41 of 42 (97%) of patients were *Helicobacter pylori* positive in at least one dental plaque sample with highest prevalence in molar region suggesting that decreased oxygen exposure favours growth of this micro-aerophilic *Helicobacter pylori*. They found that 23 of 42 (55 %) patients had *Helicobacter pylori* DNA in the saliva samples. They concluded that *Helicobacter pylori* could be the normal micro flora of adult oral cavity and also suggested that there could be no correlation between the prevalence of *Helicobacter pylori* in dental plaque samples from different locations, saliva and infection in the stomach of adult patients

Dowsett SA and Kowolik MJ [39] in 2003 reviewed the literature to assess the role of oral cavity in the transmission of *Helicobacter pylori* and the role of the oral cavity as a reservoir for gastric *Helicobacter pylori* infection by culture and PCR methods. They have analysed and found that crowded living condition, low socio-economic status and clustering of disease within family units have contributed to the high prevalence of *Helicobacter pylori* infection. They also suggest detection of *Helicobacter pylori* based on the production of large amount of urease can be accepted in the gastric detection but in the oral cavity such detection would not be possible, as several urease producing species such as Streptococcus spp., Haemophilus spp. and Actinomyces spp., also produce urease, which could potentially give a false positive results. So, rapid urease test alone cannot be recommended for definitive identification of oral *Helicobactor pylori*. They also suggest that the currently accepted 'gold standard' for the diagnosis of gastric *Helicobactor pylori* is culture, and this could provide a definitive method for the

detection of oral *Helicobacter pylori*. However, the culture of oral *Helicobacter pylori* is a complex procedure, as the oral microflora containing the fastidious *Helicobacter pylori* requires a micro-aerophilic environment and supplemented media up to seven days incubation. This may cause the overgrowth of other oral species, which in turn could inhibit the growth of *Helicobacter pylori* species. They also suggested the presence of viable coccoid forms that is unculturable by conventional techniques and which can revert back to infectious rod-shaped form under appropriate conditions could also not be detected by conventional cultures. They suggest that oral cavity could be a potential reservoir for *Helicobacter pylori* infection, which could be acquired from the stomach via a gastro-oral route and could reside in the dental plaque which are inaccessible by systemic antibiotics and could cause re-infection of gastric infections.

Czesnikiewicz-Guzik. M, Karczewska E, Bielanski W et al[4] in 2004 conducted a Polish study to determine the prevalence of *Helicobacter pylori* in the oral cavity. 100 female subjects were recruited and 13C-urea breath test was performed to determine the gastric *Helicobacter pylori* infection. The presence of the bacteria in the oral cavity was monitored by the culture from the saliva and from dental plaque. They found that 51 of 100 individuals had *Helicobacter pylori* in the stomach, 54 of 100 subjects had *Helicobacter pylori* in their saliva and 48 of 100 harboured *Helicobacter pylori* in their gingival pockets. They also found that anti-HP IgA was found in 84 of 100 individuals. 55% of subjects with stomach infection also had concomitant presence of *Helicobacter pylori* in saliva. 53% with negative Urease Breath Test revealed the presence of *Helicobacter pylori* in culture from the saliva. They also compared the presence of *Helicobacter pylori* in the saliva of patients with and without teeth and found that

presence of teeth does not affect the occurrence of *Helicobacter pylori* in the saliva. They concluded that the oral cavity contamination with *Helicobacter pylori* occurs at similar degree to that of the stomach and there was no significant correlation between the occurrence of *Helicobacter pylori* in the stomach and in the oral cavity. This indicates that the other factors, like susceptibility to infection due to acid environment in the stomach may be the major factor in gastric infection with the bacteria, while oral cavity may serve only as transient food-related contamination source without clear relation to gastric infection. They also hypothesized that continuous exposure of the dentist to the aerosols derived from the patient's oral cavity are associated with continuous exposure to *Helicobacter pylori* which could lead to gastric infection.

Tiwari SK, Khan AA, Ahmed KS et al[11] in 2005 conducted an Indian study to evaluate a non-invasive method for the rapid diagnosis to determine the prevalence of *Helicobacter pylori* in the salivary secretion of patients suffering from various gastric pathologies. 80 symptomatic gastritis patients and 20 patients with no evidence of mucosal ulcer or gastritis during gastro endoscopy were recruited for the study. 2-3 ml of saliva samples were collected along with their gastric biopsies. Three gastric biopsies were collected one each for rapid urease test, PCR assay and histological examination by modified giemsa stain for the presence of *Helicobacter pylori*. PCR amplification was done targeting the 16S rRNA with a standard in-house protocol. They found that 72 of 80 patients with symptomatic gastritis and 10 of 20 asymptomatic subjects were infected with *Helicobacter pylori* in the stomach in both histological examination and PCR assay of the gastric biopsy. 70 of 80 symptomatic subjects and 12 of 20 asymptomatic subjects had *Helicobacter pylori* DNA in the saliva samples.

Thereby, they concluded that high detection rate of *Helicobacter pylori* in saliva samples

Nao Suzuki, Masahiro Yoneda, Toru Naito et al[8] in 2008 conducted a study in Japanese population to detect the prevalence of *Helicobacter pylori* DNA in the saliva samples of patients with complaints of halitosis. 326 non-dyspeptic patients with complaints of halitosis were recruited and volatile sulphur compound levels were evaluated. The patients were subjected to organoleptic tests and gas chromatography to determine the severity of malodour. They also recorded the periodontal pocket depth, tongue coating and collected saliva for determining the presence of occult blood, *Helicobacter pylori*, Porphyromonas gingivalis, Treponema denticola and Prevotella intermedia in saliva. They detected *Helicobacter pylori* in 21 of 326 samples with highest prevalence observed in subjects between 30-39 years. They also found the methyl mercaptan, occult blood, periodontal pocket depth greater or equal to 6mm, Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia were more expressed in *Helicobacter pylori* positive patients than in *Helicobacter pylori* negative patients.

Ryuhei Yamada, Akira Yamaguchi and Koichi Shibasakil[40] in 2008 conducted a study in Japanese population to determine *Helicobacter pylori* DNA from the gastric juice, saliva and urine by nested PCR. 39 patients who underwent endoscopy at the Nippon Dental University Medical Hospital were recruited for the study. 19 patients were diagnosed with gastric ulcer, 17 patients were diagnosed with chronic gastritis and 3 patients were diagnosed with gastric cancer. Gastric juice and saliva samples were collected from all the 39 patients, but urine samples could be collected from 26 patients only. They found that *Helicobacter*

pylori DNA was detected in gastric juice from all 39 patients, *Helicobacter pylori* DNA was detected in 28 of 39 patients in their saliva sample and in the urine samples of 13 of the 26 patients. They have detected *Helicobacter pylori* DNA from gastric juice, saliva and urine by nested PCR using two pairs of primers, EHC-U/EHC-L and ET-5U/ET-5L, targeting 417-bp and 230-bp DNA of *Helicobacter pylori* respectively. They also convey that since *Helicobacter pylori* is not necessarily distributed evenly in saliva, and since the PCR method is so sensitive that it can detect DNA in trace amounts in samples, the failure to detect *Helicobacter pylori* DNA should not necessarily lead to the conclusion that saliva is negative *Helicobacter pylori*, and further it is important to consider sampling errors and contamination in the interpretation of results. They also conveyed that their literature search failed to find a report describing *Helicobacter pylori* DNA detection in urine. They proposed a pathway of *Helicobacter pylori* DNA entry into the blood circulation through the injured gastric mucosa from which gets absorbed into small intestine reaches the liver via the portal vein and then released into the blood as *Helicobacter pylori* DNA, which subsequently gets filtered through the renal glomeruli and excreted in the urine. They also feel that *Helicobacter pylori* infection in the urinary bladder or kidney may also cause detection of the *Helicobacter pylori* DNA in the urine. They concluded that *Helicobacter pylori* DNA in gastric juice was related to the detection of *Helicobacter pylori* DNA in the saliva but not to the detection of *Helicobacter pylori* DNA in the urine.

Denise G Silva, Roy H Stevens, Jacyara MB Macedo et al[41] in 2009 conducted a study in a Brazilian population of 62 patients for the detection of *Helicobacter pylori* and its virulent cagA gene in the oral cavity of individuals with upper

gastric disease. The study subjects were divided into 2 groups. One group consisted of 30 patients with symptoms of gastric disease and the other group consisted of 32 controls. Saliva, dental plaque and antral biopsy samples were collected from the subjects and the presence of *Helicobacter pylori* was analysed by polymerase chain reaction targeting the 16S ribosomal and cagA genes. Control group showed absence of *Helicobacter pylori* in the saliva and dental plaque. The patients with symptoms of gastric disease showed positivity for *Helicobacter pylori* in 16 of the 30 saliva samples and 11 of 30 dental plaque samples. cagA gene was detected in 13 of 30 antral biopsies, 7 of 16 positive saliva samples, 3 of 11 positive dental plaque samples. 18 subjects with gastric disease were positive for *Helicobacter pylori* in both oral and gastric biopsy samples and 8 of those 18 were positive for cagA *Helicobacter pylori* DNA. Thus they concluded that dental plaque and saliva may serve as temporary reservoir for *Helicobacter pylori* and its virulent cagA variant in subjects with symptoms of gastric disease.

Luigina Cellini, Rossella Grande, Luciano Arteseet al[7] in 2010 conducted an Italian study to determine the prevalence of *Helicobacter pylori* in the oral cavity. 19 patients who had showed positivity with urease breath test were recruited. Saliva samples, mucosal biopsies of middle-distal oesophagus, gastric antrum and fundus was collected and found that *Helicobacter pylori* was detected in the 18 of 19 cultured samples. The nested PCR of the saliva samples found that *Helicobacter pylori* DNA was found in all 19 patients. Their study also stated that oesophagus and oral cavity could be extra-gastric reservoirs of *Helicobacter pylori*. They concluded that by understanding the route of *Helicobacter pylori* transmission an effective disease management can be developed.

Myriam Lucrecia Mediana, Marcelo-Gabriel Medina, Graciela-Teresita Martin et al[9] in 2010 conducted a study on Argentanian population to determine the presence of *Helicobacter pylori* in the oral cavity and gastric mucosa of patients with digestive pathologies. 98 patients consisting of 43 patients with gastric symptoms and 55 asymptomatic controls were recruited for the study. They collected saliva samples, dental plaque in all the patients. Gastric biopsy was performed in patients with symptoms of digestive pathologies. In the oral samples, *Helicobacter pylori* detection was carried by polymerase chain reaction method. *Helicobacter pylori* was present in 18 of 98 patients. They also found presence of *Helicobacter pylori* in gastric biopsy in 38 of 43 patients. They also found that 15 of 43 patients with gastric symptoms had both positivity for *Helicobacter pylori* in saliva and gastric mucosal biopsy. They concluded that presence of *Helicobacter pylori* in patients with digestive pathologies is more frequently seen in those associated with gingivoperiodontal diseases. Gingivoperiodontal diseases can inhibit the complete eradication of the bacterium and act as a risk factor for gastro-intestinal re-infection after treatment.

Denise G Silva, Eduardo MB Tinoco, Gifone A Rochaet al[16]in 2010 conducted a study in a Brazilian population to investigate the presence of *Helicobacter pylori* in the stomach and the oral cavity. 30 patients who were to undergo upper endoscopy were recruited and antral biopsy was performed. Saliva and dental plaque samples were collected. Nested PCR targeting 16S rDNA products of 400 base pairs was done to ascertain the presence of *Helicobacter pylori* bacteria. They also evaluated the presence of vacA gene by single step PCR. They found that 24 of the 30 gastric biopsies were positive for 16S r RNA gene. They also found the 9 of the 30 saliva samples and 6 of the 30 dental plaque samples were

positive for the *Helicobacter pylori* 16S rRNA gene. They suggested that the variation in many studies on the detection of *Helicobacter pylori* could be due to differences in the prevalence of gastric *Helicobacter pylori* infection or in the accuracy of the methods used. They also suggested that prevalence of infection increases with increasing age and concluded that age of the patient is an important factor in analyzing the study. They concluded that even though *Helicobacter pylori* do not persistently colonize and cause oral diseases, they could participate in the transmission of the infection by oral route. *Helicobacter pylori* may transiently be present in oral cavity of patient with gastric infection and can serve as a likely source for transmission of infection by oral route. They also suggest that detection of *Helicobacter pylori* by PCR at oral sites should be interpreted with caution as many other oral microbial floras arephylo-genetically related to *Helicobacter pylori.*

DENTAL PLAQUE IN *HELICOBACTER PYLORI* INFECTION

Anne-Marie H. Nguyen, Fouad A. K. El-Zaatari and David Y. Grahaml[42], 1995 in a critical review of literature found that *Helicobacter pylori* in the stomach are responsible for the pathogenesis of duodenal ulcer disease, gastric ulcer disease, gastric carcinoma and primary gastric B-cell lymphoma. They found that even though culture is the gold standard method for the detection, polymerase chain reaction is the powerful tool for the detection with good sensitivity, specificity and rapidity. Most polymerase chain reaction analysis was directed at urease gene and 16S ribosomal RNA gene. It was found that person to person, faecal-oral and oral-oral routes have contributed for the transmission of the infection. It was also found that *Helicobacter pylori* had also been detected from the oral cavity of patients with no *Helicobacter pylori* infection in the

stomach. Thus, they suggested that antimicrobial therapy failure in *Helicobacter pylori* infection could be due to its presence in the oral cavity which could re-infect the stomach. Thus oral cavity could contribute immensely for the spread of infection.

Riggio MP and Lennon A[19] in 1999 conducted a study in an Irish population of 29 patients with chronic adult periodontitis with at least 3 periodontal pockets more than 5 mm and with no symptoms of gastritis or peptic ulcer disease sub gingival plaques were collected using a single stroke of sterile curette. Polymerase chain reaction was done targeting 16S ribosomal RNA gene of *Helicobacter pylori* to amplify a 295 base pair DNA fragment and confirmed by southern blot hybridisation. 2-4 sub gingival plaque from different periodontal pockets were analysed from each patient and *Helicobacter pylori* DNA was detected in 24 of 73 samples analysed, 11 of 29 patients harboured *Helicobacter pylori* in at least one analysed site and thereby concluded that subgingival plaque could be an extragastric reservoir of *Helicobacter pylori* infection

Alejandra Berroteran, Marianella Perrone, Maria Correnti et al[10]in 2002 conducted a study in Venezuelan population of 32 dyspeptic patients and 20 asymptomatic subjects. Dental plaque was collected from all patients and 2 antral biopsy specimens from 32 dyspeptic patients. They performed a polymerase chain reaction assay targeting urease gene for *Helicobacter pylori* and found that 24 out of 32 gastritis patients had *Helicobacter pylori* in the gastric specimen and 12 out of 32 gastritis patients had it in dental plaque. 7 out of 12 patients with positive *Helicobacter pylori* in dental plaque also had *Helicobacter pylori* in the gastric mucosa. They also found that prevalence of *Helicobacter pylori* infection

was significantly higher in females and in patients who were in the age group of 40-59 years. They also found that non-smokers and non-drinkers had a higher PCR positivity rate for *Helicobacter pylori* in both dental plaque and gastric biopsies. They concluded that oral cavity can be an extragastric reservoir and oral secretion can acts as a means of transmission of *Helicobacter pylori*. They also conveyed that this micro-organism in dental plaque might be a risk factor for gastro-intestinal re-infection and ulcer relapse after antibiotic therapy.

Mohammad Taghi Chitsazi, Ebrahim Fattahi, Ramin Mostofi Zadeh Farahani et al[21] in 2006 conducted a study in an Iranian population of 88 subjects with and without *Helicobacter pylori* infection. Rapid urease test were performed on specimens from gastric antrum and dental plaque collected from mandibular first and second molar region. *Helicobacter pylori* was detected by rapid urease test, if the colour changed from yellow to red within 1 hour. *Helicobacter pylori* were detected in 34.1% of dental plaque specimens. The prevalence of *Helicobacter pylori* in dental plaque was 31.8% and 36.4% in patients with and without gastric infection respectively. They also found that *Helicobacter pylori* presence in stomach and dental plaque was more in males than in females. Thus, they concluded that there was no association between *Helicobacter pylori* in dental plaque and stomach and detection of *Helicobacter pylori* in oral cavity could be of no diagnostic value for the gastric infection.

S. Zaric, B. Bojic, LJ. Jankovic et al[43] in 2009 conducted a study in a Serbian population of 98 patients with upper digestive tract infection. Endoscopy was performed and oral hygiene indices were recorded. The 98 patients were divided into 3 groups based on the presence of *Helicobacter pylori* in gastric samples and

oral cavity and the mode of treatment. The modes of treatment included triple eradication therapy alone and/ or triple eradication concomitant periodontal therapy. They found that patients who received both triple eradication therapy and periodontal therapy showed a higher eradication rate and also found that deeper periodontal pockets could harbour *Helicobacter pylori* and provide a micro-aerophilic environment for its colonization. Thereby, they concluded that the presence of periodontitis could be a risk factor for the recurrence of *Helicobacter pylori* infection. Triple eradication drug therapy and proper periodontal treatment improves the outcome and prognosis of the infection recurrence**.**

Amir Eskandari, Ali Mahmoudpour, Nader Abolfazli et al[23] in 2010 conducted a study in an Iranian population of 67 chronic periodontitis patients. Dental plaque samples were removed from an anterior and a posterior tooth using a sterile periodontal curette. 23 of these patients were diagnosed for *Helicobacter pylori* gastritis by endoscopy and rapid urease test. Polymerase chain reaction amplification was done for detection of *Helicobacter pylori* in clinical specimen. They found that *Helicobacter pylori* was present in 59% of patients with periodontitis and there was an association between the presence of *Helicobacter pylori* in the dental plaque and the presence of gastritis. Thus, they conclude that *Helicobacter pylori* infected dental plaque can act as a source for re-infection. So, professional oral hygiene measures should be performed supplementary to the conventional antibiotic treatment for *Helicobacter pylori*.

Lucas Trevizani Rasmussen, Roger William de Labio, Luciano Lobo Gatti[22] et al in 2010 conducted a study in a Brazilian population of 78 dyspeptic patients.

Gastric biopsy, saliva samples and dental plaques were collected. The DNA was extracted from these samples and the presence of *Helicobacter pylori* was evaluated using polymerase chain reaction and southern blotting methods. They found that *Helicobacter pylori* was frequently seen in stomach of patients with gastritis and there was *Helicobacter pylori* in both the gastric biopsy and oral cavity. Thus they concluded that a relationship exists between gastric infection and presence of *Helicobacter pylori* in the oral cavity. Thus the presence of *Helicobacter pylori* in the oral cavity suggests that oral cavity provides a safe reservoir for the bacterium and thereby offers potential relation for the gastric re-infection.

HELICOBACTER PYLORI **IN ADENOID TISSUE AND IN UPPER RESPIRATORY TRACT INFECTION**

Meltem Yalinay Cirak, Ali Ozdek, Dicle Yilmaz et al[24] in 2003 conducted a study in a Turkish population to determine the presence of *Helicobacter pylori* in the adenotonsillectomy specimens by PCR. 23 patients who had undergone a tonsillectomy or adenoidectomy procedure, core biopsy specimens from tonsil and adenoid tissues were collected. The DNA was extracted and the DNA was amplified using Polymerase chain reaction for 16S rRNA gene and Cag A gene. The analysis found that 7 of 23 patients were positive for *Helicobacter pylori* DNA and CagA gene was detected in 5 of 7 patients. This presence of *Helicobacter pylori* in the mouth supports the potential spread of the organism via interpersonal route. CagA protein is a surface immune dominant antigen found to have close association with the production of vacuolating cytotoxin. They felt that polymerase chain reaction is the best tool for diagnosis as culture is difficult to perform. They also suggested that the accuracy of Polymerase chain reaction

can be affected by the choice of primers, target DNA, bacterial density in sample, specimen preparation and variation in laboratory protocol. They concluded that tonsil and adenoid tissue could act as an ecological niche in the mouth for transient or permanent colonization and possible oral-oral transmission.

Anne Pitkaranta, Kaija-Leena Kolho and Hilpi Rautelin[44] in 2005 conducted a study to examine the presence of *Helicobacter pylori* infection in children with recurrent upper respiratory infection. 20 children from the refugee families of African origin admitted in the Helsinki University Central Hospital, Helsinki, Finland with frequent respiratory tract infection and recurrent acute otitis media were recruited. Adenoidectomy was performed and middle ear effusion, serum samples and faecal samples were collected. The samples were subjected to culture, serologic test and faecal antigen test, respectively. They found that only 20% of children with frequent respiratory tract infection and recurrent acute otitis media were *Helicobacter pylori* positive on serological tests and/or faecal antigen detection. They also found that all the *Helicobacter pylori* culture showed negativity suggesting that no association existed between *Helicobacter pylori* infection and frequent respiratory tract infection and recurrent acute otitis media.

ABSENCE OF *HELICOBACTER PYLORI* WITHIN THE ORAL CAVITY

Sigmund Krajden, Milan Fuksa and Joe Anderson et al [20] in 1989 conducted a study in Canadian population to evaluate the presence of *Helicobacter pylori* within the oral cavity. 71 patients with clinical symptoms of gastritis were recruited. Saliva, dental plaques samples were collected before endoscopy and gastric biopsy was done during endoscopy. They cultured the collected saliva, dental plaque and antral biopsy specimen in a selective non-inhibitory Skirrow

medium and found that 29 of 71 antral biopsies cultured *Helicobacter pylori*, 1 of 71 dental plaque cultured *Helicobacter pylori* and none of the saliva sampled could culture *Helicobacter pylori*. Therefore, they concluded that neither saliva nor dental plaque could be implicated as a significant reservoir of *Helicobacter pylori* organism.

Brenda J Olivier, Robert P Bond, Walda B van Zylet al [45]in 2006 conducted a study in South African population to evaluate the oral cavity as a reservoir of *Helicobacter pylori* infection. 79 rural healthy individuals were recruited for the study and dental plaque samples were collected from the oral cavity. Endoscopy was performed and two gastric biopsies from antrum and the corpus of the stomach were collected. A single step PCR directed towards the urease AB gene, a hemi nested PCR directed towards the phosphoglucosaminemutase (glmM) gene of H. pylori were performed on dental plaque and biopsy samples. A third nested PCR directed towards the 860-bp DNA region of *Helicobacter pylori* was performed on dental plaque samples. They found that the hemi-nested PCR showed greater sensitivity and specificity than the single-step PCR for the DNA extracted from biopsy samples, but no positive results were obtained from any of these PCR methods on dental plaque samples. They concluded that their study indicated that the oral cavity does not favour prolonged colonization of *Helicobacter pylori* in healthy black South African population with a high prevalence of infection, indicating that another mode of transmission exists in which the oral cavity is unlikely to contribute to the spread of this organism.

Silva Rossi-Aguiar VP, Navarro-Rodriguez T, Mattar R et al [46] in 2009 conducted a study in a Brazilian population of 43 subjects with epigastric pain

syndrome, gastrointestinal endoscopy was done. Rapid urease test and urease breath test were performed to confirm the presence of *Helicobacter pylori* infection in the stomach. Sterile cotton swabs were used to collect saliva from oral mucosa. Posterior third of the dorsum of tongue and supra gingival plaque was collected using a sterile curettes. Sub gingival plaque was collected by inserting a sterile paper point in patients with periodontitis. Polymerase chain reaction was performed to amplify regions of urease, cagA and vac A genes. They found that 30 subjects harboured *Helicobacter pylori* in the stomach but could not be possible to detect *Helicobacter pylori* in oral samples using urease and vacA genotype could not be characterized. Thus, they conclude that oral cavity may not be a reservoir for *Helicobacter pylori* in the epigastric pain syndrome subjects.

MUCOSAL ADHESION OF *HELICOBACTER PYLORI*

Jos A. Bosch, Eco J. C. de Geus, Toon J. M. Ligtenberget al [47] in 2000 conducted a study in a Dutch population of 18 undergraduate volunteers. Their blood pressure, impedance cardiogram and electrocardiogram were recorded and saliva was collected during an acute stressor exposure to detect glycoprotein MUC5B-linked sulfo-Lewis and total salivary protein. They found that during stress exposure, there was an increased anxiety, stroke volume and a decreased heart rate. They also found that salivary sulfo-Lewis concentration, Sulfo-Lewis output, Sulfo-Lewis/Total protein ratio and saliva MUC5B mediated adherence of *Helicobacter pylori* increased during stressful condition. Thus they concluded that stress could increase bacterial adherence to the mucosal surface of the oral cavity and could act as a contributing factor for the transmission and recurrence of *Helicobacter pylori* infection.

HELICOBACTER PYLORI AND RECURRENT APHTHOUS STOMATITIS

Junia Maria Netto Victoria, EvanguedesKalapothakis, Jeane de Fatima Correia Silva[48] et al in 2003 conducted a study in a Brazilian population of 36 subjects with recurrent aphthous stomatitis and 48 healthy volunteers. Oral mucosa swabs were collected from each subject with recurrent aphthous stomatitis from the lesion before the third day of onset and also from the contralateral intact mucosa. The lesion presented with well circumscribed margins and was surrounded by an erythematous halo. The swabs from the healthy volunteers were collected from the labial mucosa, floor of mouth and tongue. Nested polymerase chain reaction was performed and found that no association was found between the recurrent aphthous stomatitis lesions and the presence of *Helicobacter pylori*. 14 out of 36 patients with recurrent aphthous stomatitis presented with *Helicobacter pylori* DNA in the lesion or on the contralateral mucosa and 16 out of 48 healthy volunteers had *Helicobacter pylori* DNA. Thus, they concluded that *Helicobacter pylori* did not contribute to the development of recurrent aphthous stomatitis.

HELICOBACTER PYLORI AND CANCER

Chun-Ying Wu, Ken N. Kuo, Ming-Shiang Wu et al [49] in 2009 conducted a study in a Taiwanese cohort study of 80,255 patients who were hospitalized for peptic ulcer disease between 1997 and 2004 and received *Helicobacter pylori* eradication therapy. They were divided into early (within 1 year) and late (after 1 year) eradication cohorts. Standardized incidence ratios (SIRs) and hazard ratios (HRs) were determined and found that even though there was no significant difference between early eradication cohorts and the general population, increased risk of cancer was seen in late eradication cohorts. They also found that the non

steroidal anti-inflammatory drugs and early eradication were independent protective factors for gastric cancer. Thus, they concluded that *Helicobacter pylori* eradication is associated with decreased risk of development of gastric cancer in patients with peptic ulcer disease.

CANDIDA IN ORAL CAVITY

Patricia Monteiro Ribeiro, Fernando Bacal, Cristiane Yumi Koga-Ito et al [50] in 2010 conducted a study in Brazilian population of 50 patients with orthotopic heart transplantation and 50 patients who were not subjected to any kind of transplantation at the University of Sao Paulo medical School's Heart Institute. Oral rinse of 10 ml Phosphate buffered saline was collected, centrifuged for 10 minutes and the deposits were used for sampling. 0.1ml of this deposit was cultured in Sabouraud Dextrose Agar with chloramphenicol and in CHROMagar. They found that counts of yeast in the subjects with orthotopic heart transplantation were higher than in subjects without orthotopic heart transplantation. *Candida* yeast counts were higher in the heart transplant recipients followed by *Candida glabrata* and *Candida tropicalis*. Interestingly, *Candida dubliniensis* was not found in both these groups.

Safia A. Al-Attas and Soliman O. Amro[27] in 2010 conducted a study in a Saudi Arabian population of 150 diabetic patients and 50 healthy controls to investigate oral Candidal colonization, strain diversity, anti-fungal susceptibility and the influence of local and systemic host factors on Candidal colonization in adult diabetics. They collected two salivary samples using the oral rinse sampling method. One of the sample was to determine the salivary flow rate and determination of pH and the other sample was for the Candidal colonization

assessment. They found that Candidal carriage was higher in the diabetic patients than in the controls and also showed higher resistance to azole antifungals. Thus, they concluded that the oral Candidal colonization was significantly associated with glycemic control, type of diabetes and salivary pH.

Radhika Sashikumar and Ranganathan Kannan[28]in 2010 conducted a study in an Indian population of 100 adults with type 2 diabetes and 50 without diabetes. These patients were grouped into three groups. Group I composed of 50 patients with controlled diabetes, group II composed of 50 patients with uncontrolled diabetes and group III composed of 50 non-diabetic subjects. 2ml peripheral venous blood was collected from every patient and random non fasting plasma glucose levels were measured using glucose-oxidase method and glycosylated haemoglobin (HbA1c) levels were measured using the ion-exchange resin method. Saliva samples for the estimation of Candidal colony forming unit was collected by oral rinse technique with 10ml sterile phosphate buffered saline. The rinse was centrifuged at 1,700 rpm for 10 minutes, supernatant was discarded and 0.001µl inoculating loop was used to spread the samples onto the Sabouraud dextrose agar plates supplemented with chloramphenicol (10mg/ml). Colony forming units were counted manually and the number was multiplied by 1000 and expressed as CFU/ml. They found that salivary glucose levels were significantly higher in diabetics than non-diabetics and a positive correlation existed between the salivary and plasma glucose levels. Candidal colony forming units were significantly higher in diabetic subjects with a positive correlation with the salivary glucose levels. Thus they conclude that the salivary glucose concentration is a useful non invasive tool for monitoring the glycemic control in diabetic patients and any increase in salivary glucose levels implies increased prevalence of oral *Candida*.

HELICOBACTER PYLORI AND *CANDIDA*

Kalogeropoulos NK and Whitehead R[30] in 1988 conducted an Australian study in 247 biopsy specimens of patients with various gastrointestinal symptoms. *Campylobacter-like* organisms were detected in 57 of 102 (56%) gastric ulcers, 11 of 12 (92%) acute inflammation, 1 of 6 (17%) chronic gastritis, 5 of 5 (100%) gastric erosions with gastritis, 3 of 5 (60%) gastric erosions without gastritis, 5 of 13 (39%) mild superficial gastritis, 2 of 36 (6%) normal gastric mucosa, 4 of 20 (20%) duodenal ulcers, 12 of 64 (19%) Barrett's oesophagus which rose to 6 of 25 (24%) in the presence of ulcerations. Oral Candidiasis was present in 20 of 78 (26%) of gastric ulcers, 6 of 25 (24%) of Barrett's Oesophagus and 9 of 78 (12%) cases with gastric ulcer specimens had both *Candida* and *Campylobacter-like organisms*. Thus, they concluded that the presence of gastrointestinal lesion in some way may promote the colonization of *Campylobacter like* organisms and *Candida*.

Ali-Hatef Salmanian, Faridah Siavoshi, Fereshteh Akbari et al [25], 2008 conducted a study of 13 yeast isolates obtained from 43 dyspeptic patients, showed the presence of non culturable forms of bacterium like bodies (BLBs) of *Helicobacer pylori* within the yeast vacuoles by targeting vacA s1s2 and ure AB genes using polymerase chain reaction. They also suggested that an endo-symbiotic relationship exists between the bacteria and the yeast, (*Candida*) in the oral cavity. They considered that this relationship might be responsible for the persistence of *Helicobacter pylori* in the oral cavity and may also contribute to re-inoculation of stomach by bacterium and spread of this infection.

E. Karczewska, I. Wojtas, E Sitoet al [29] in 2009 conducted a study in a Polish population of 158 patients with symptoms from upper gastrointestinal tract, 97 patients were found positive for *Helicobacter pylori* in gastric mucosa, 27 patients were positive for *Candida*. Quantitative analysis showed pathologically insignificant number of *Candida* ($<10^3$ CFU/ml). They also found that duodenal ulcers are higher in condition where *Helicobacter pylori* is present alone and in case of concurrent occurrence of *Helicobacter pylori* and *Candida*, gastric ulcer is more common. They also found that patients infected with Cag A, Vac A s1 strain can develop peptic ulcer diseases more frequently

Results

The study group comprised of 70 subjects, 60 of whom were symptomatic gastritis and 10 were asymptomatic controls. The symptomatic gastritis group had undergone endoscopy and the gastric biopsy specimen had been examined histopathologically, for the presence of *Helicobacter pylori*. The subjects were divided into 3 groups.

Group I comprised of 30 subjects with *Helicobacter pylori* in their gastric mucosa.
Group II comprised of 30 subjects without *Helicobacter pylori* in their gastric mucosa.
Group III comprised of 10 asymptomatic controls.

In our study comprising 70 subjects, we found that 52 (74.3%) were males and 18 (25.7%) were females. Among the males (n=52), 12 (23.1%) were less than 30 years of age, 25 (48.1%) were between 30 -50 years and 15 (28.8%) were above 50 years of age. Among the females (n=18), 5 (27.8%) were less than 30 years of age, 9 (50%) were between 30-50 years and 4 (22.2%) were above 50 years of age. In the overall study subjects (n=70), 17 (24.3%) were less than 30 years of age, 34 (48.6%) were between 31-50 years and 19 (27.1%) were above 50 years of age **[Table 1, Graph 1].**

Among the subjects with *Helicobacter pylori* in their gastric mucosa (Group I) n=30, 23 (76.7%) were males and 7 (23.3%) were females. Among males (n=23), 6 (26.1%) were less than 30 year of age, 13 (56.5%) were between 31-50 years of age and 4 (17.4%) were above 50 years of age. Among females (n=7), 5(71.4%) were between 31-50 years and 2 (28.6%) were above 50 years of age. Among the subjects without *Helicobacter pylori* in their gastric mucosa

(Group II) n=30, 24 (80%) were males and 6 (20%) were females. Among males (n=24), 3 (12.5%) were less than 30 year of age, 10 (41.7%) were between 31-50 years of age and 11 (45.8%) were above 50 years of age. Among females (n=6), 4 (66.7%) were between 31-50 years and 2 (33.3%) were above 50 years of age. Among the control group (n=10), 5 (50%) were males and 5 (50%) were females. Among the males (n=5), 3 (60%) were less than 30 years of age and 2 (40%) were between 31-50 years. Among the females (n=5), all were less than 30 years of age **[Table 2, Graph 2].**

Among subjects with *Helicobacter pylori* in their saliva (n=11), 9 (81.8%) were males and 2 (18.2%) were females. Among males (n=9), 2 (22.2%) were less than 30 years, 5 (55.6%) were between 31-50 years and 2 (22.2%) were above 50 years. Among females (n=2), all were between 31-50 years of age. Among this group (n=11), 2 (18.2) were less than 30 years of age, 7 (63.6%) were between 31-50 years and 2 (18.2%) were above 50 years of age. Among subjects without *Helicobacter pylori* in their saliva (n=59), 43 (72.9%) were males and 16 (27.1%) were females. Among males (n=43), 10 (23.3%) were less than 30 years, 20 (46.5%) were between 31-50 years and 13 (30.2%) were above 50 years. Among females (n=16), 5 (31.3%) were less than 30 years of age, 7 (43.8%) were between 31-50 years of age and 4 (25%) were above 50 years of age. Among this group (n=59), 15 (25.4%) were less than 30 years of age, 27 (45.8%) were between 31-50 years and 17 (28.8%) were above 50 years of age. **[Table 3, Graph 3]**

Among subjects with Candida in their saliva (n=25), 17 (68%) were males and 8 (32%) were females. Among males (n=17), 6 (35.3%) were less than 30

years, 8 (47.1%) were between 31-50 years and 3 (17.6%) were above 50 years. Among females (n=8), 3 (37.5%) were less than 30 years, 4 (50%) were between 31-50 years of age and 1 (12.5%) was above 50 years of age. Among this group (n=25), 9 (36%) were less than 30 years of age, 12 (48%) were between 31-50 years and 4 (16%) were above 50 years of age. Among subjects without *Candida* in their saliva (n=45), 35 (77.8%) were males and 10 (22.2%) were females. Among males (n=35), 6 (17.1%) were less than 30 years, 17 (48.6 %) were between 31-50 years and 12 (34.3%) were above 50 years. Among females (n=10), 2 (20%) were less than 30 years of age, 5 (50%) were between 31-50 years of age and 3 (30%) were above 50 years of age. Among this group (n=45), 8 (17.8%) were less than 30 years of age, 22 (48.9%) were between 31-50 years and 15 (33.3%) were above 50 years of age **[Table 4, Graph 4].**

In patients with symptoms of gastritis with less than 30 years of age (n=9), 6 of 9 (66.7%) patients showed the presence of *Candida* in the saliva samples and 3 of 9 (33.3%) patients showed the absence of *Candida* in the saliva samples. In patients with symptoms of gastritis within 30 and 50 years of age (n=32), 12 of 32 (37.5%) patients showed the presence of *Candida* in the saliva samples and 20 of 32 (62.5%) patients showed the absence of candida in the saliva samples. In patients with symptoms of gastritis more than 50 years of age (n=19), 4 of 19 (21.1%) patients showed the presence of *Candida* in the saliva samples and 15 of 19 (78.9%) patients showed the absence of *Candida* in the saliva samples **[Table 5, Graph 5].**

When a correlation coefficient was done between various variables in the study group. Age vs Oral *Candida*, the correlation coefficient was - . 0.296 (p

value = 0.022*) Age vs Oral candida in gastric *Helicobacter pylori* group , the correlation coefficient was – 0.211 (p value=0.26) Age vs Oral *candida* in gastric *Helicobacter pylori* negative group , the correlation coefficient was – 0.331 (p value=0.074). Age vs Oral *candida* in control group was – 0.327 (p value= 0.356). Gastric *Helicobacter pylori* vs Salivary *Helicobacter pylori*, the correlation was 0.129 (p value=0.325).On correlating Salivary *Helicobacter pylori* vs Oral *candida*, the correlation coefficient was 0.003 (p value=0.982). Gastric *Helicobacter pylori* vs Oral *candida*, the correlation coefficient was 0.208 (p=0.112) **[Table 6]**

Among the symptomatic gastritis patients with *Helicobacter pylori* in gastric biopsy specimen (n=30), 7 of 30 (23.3%) patients showed that H. pylori was present in saliva samples and 23 of 30 (76.7%) showed the absence of *Helicobacter pylori* in saliva samples by polymerase chain reaction. Among the symptomatic gastritis patients without *Helicobacter pylori* in gastric biopsy specimen (n=30), 4 of 30 (13.3%) patients showed that *Helicobacter pylori* was present in saliva samples and 26 of 30 (86.7%) showed the absence of *Helicobacter pylori* in saliva samples by polymerase chain reaction. The odds ratio for gastritis and Salivary *Helicobacter pylori* was 1.98 (p=0.506, 95% CI: 0.51-7.61) **[Table 7]**

Among patients with salivary candidal carriage (n=22), 14 of 22 (63.6%) showed the presence of *Helicobacter pylori* in their gastric mucosa and 8 of 22 (36.4%) did not show *Helicobacter pylori* in their gastric mucosa. Among patients who did not show any salivary candidal carriage (n=38), 16 of 38 (42.1%) had *Helicobacter pylori* in their gastric mucosa and 22 of 38 (57.9%) did not have

Helicobacter pylori in their gastric mucosa. The odds ratio for gastritis and Oral candida in the oral cavity was 2.4 (p=0.506, 95% CI: 0.82-7.1) **[Table 8]**

Among symptomatic patients with *Helicobacter pylori* in the gastric mucosa (n=30), both salivary *Helicobacter pylori* and *candida* co-existed together in only 4 (13.3%) subjects but such co-existence did not appear in 26 (86.7%). Among symptomatic patients without *Helicobacter pylori* in the gastric mucosa (n=30) none of the patients had both salivary *Helicobacter pylori* and *Candida* together. The odds ratio for gastritis and Oral *Candida* and salivary *Helicobacter pylori* was 8.22 (p=0.038*, 95% CI: 1.1-61.495**)[Table 9]**

The mean CFU in Group I was 10,857 ±14,570.03

The mean CFU in Group II was 10,625 ± 16,238.73

The mean CFU in Group III was 39,333.33 ±52,548.39

The mean CFU in less than 30 years was 15,777.78 ±31,732.38

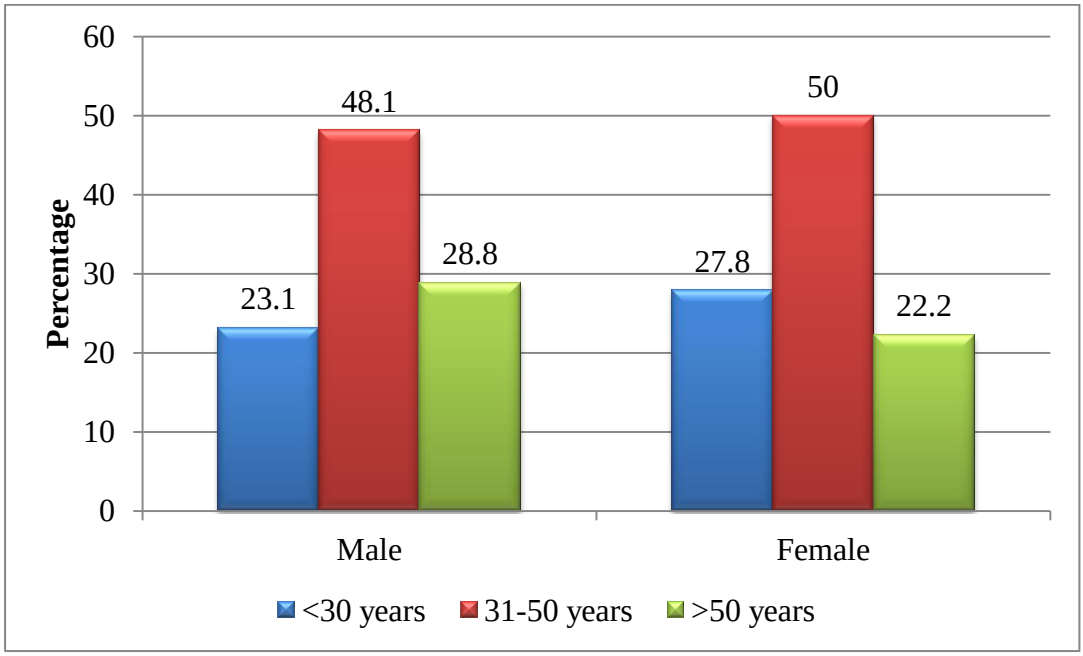
The mean CFU between 30 and 50 years was 10,916 ± 15347.24

The mean CFU above 50 years was 14,200 ±22,621.

Tables & Graphs

Table 1: AGE AND GENDER DISTRIBUTION OF ALL PATIENTS (N=70)

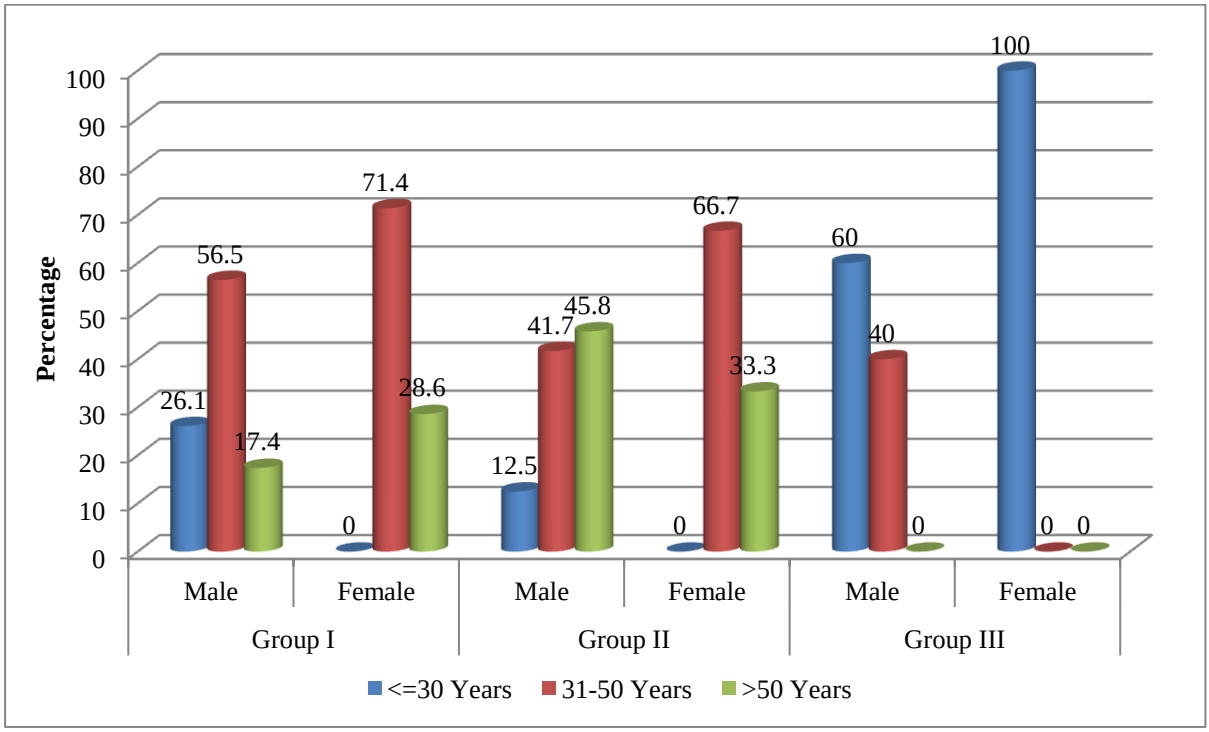
	Gender				Total		p value
	Male (n=52)		Female (n=18)				
	n	%	n	%	n	%	
<=30 years	12	23.1	5	27.8	17	24.3	0.840
31-50 years	25	48.1	9	50	34	48.6	
>50 years	15	28.8	4	22.2	19	27.1	



Graph 1: AGE AND GENDER DISTRIBUTION OF ALL PATIENTS (N=70)

Table 2: AGE AND GENDER DISTRIBUTION BETWEEN THE STUDY GROUPS (N=70)

Age Groups	Group I				Group II				Group III			
	Male		Female		Male		Female		Male		Female	
	n	%	n	%	n	%	n	%	n	%	n	%
<=30	6	26.1	-	-	3	12.5	-	-	3	60	5	100
31-50	13	56.5	5	71.4	10	41.7	4	66.7	2	40	-	-
>50	4	17.4	2	28.6	11	45.8	2	33.3	-	-	-	-
Total	23	76.7	7	23.3	24	80	6	20	5	50	5	50
p value	0.307				0.450				0.444			



Graph 2: AGE AND GENDER DISTRIBUTION BETWEEN THE STUDY GROUPS (N=70)

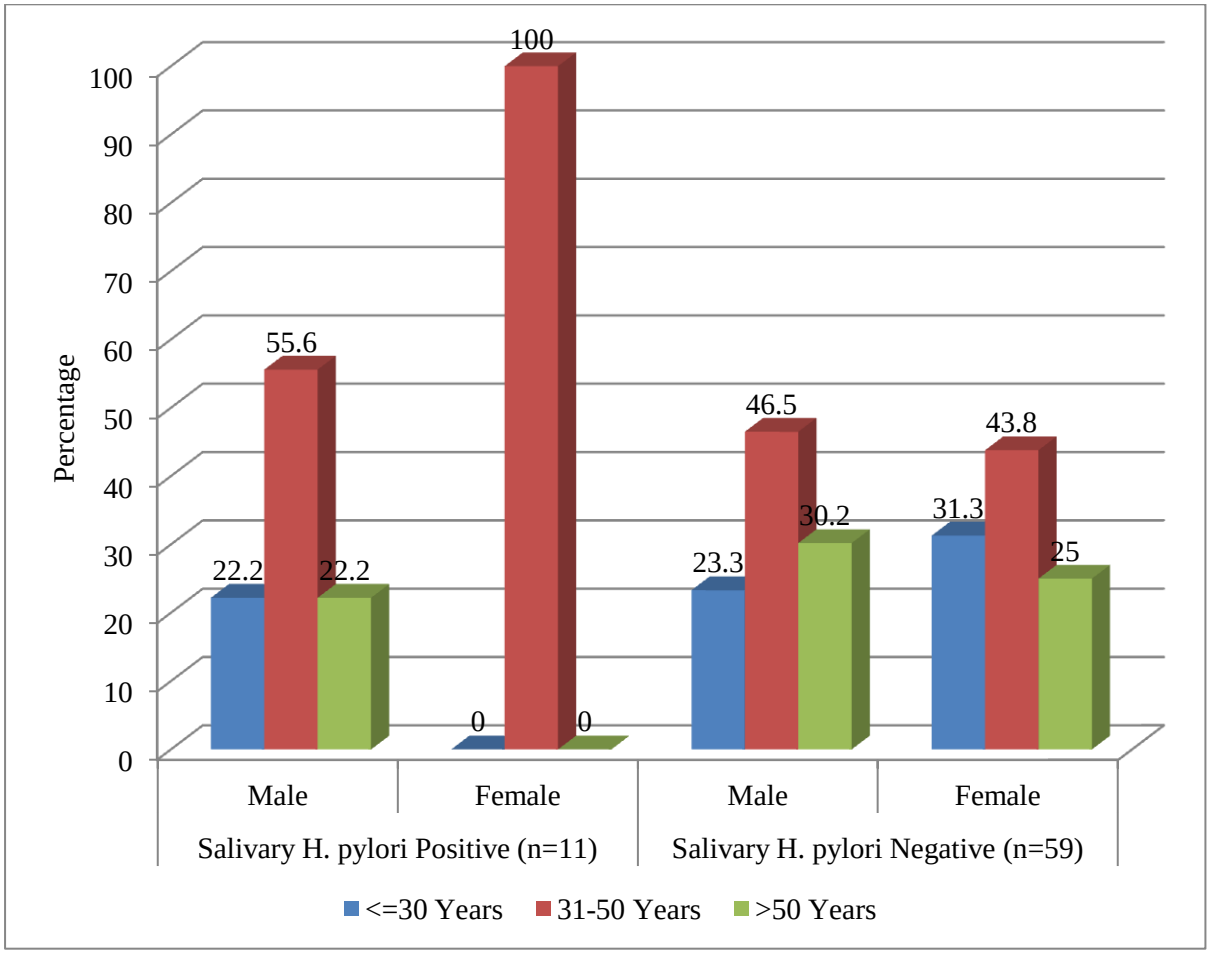
Group I: Symptomatic gastritis subjects with Helicobacter pylori in gastric mucosa

Group II: Symptomatic gastritis subjects without Helicobacter pylori in gastric mucosa

Group III: Asymptomatic normal controls

Table 3: AGE AND GENDER DISTRIBUTION BETWEEN SALIVARY *Helicobacter pylori* POSITIVE AND SALIVARY *Helicobacter pylori* NEGATIVE SUBJECTS (N=70)

Age Groups	Salivary H. pylori Positive (n=11)						Salivary H. pylori Negative (n=59)					
	Male		Female		Total		Male		Female		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
<=30	2	22.2	-	-	2	18.2	10	23.3	5	31.3	15	25.4
31-50	5	55.6	2	100	7	63.6	20	46.5	7	43.8	27	45.8
>50	2	22.2	-	-	2	18.2	13	30.2	4	25	17	28.8
Total	9	81.8	2	18.2	11	100	43	72.9	16	27.1	59	100
p value	0.497						0.809					

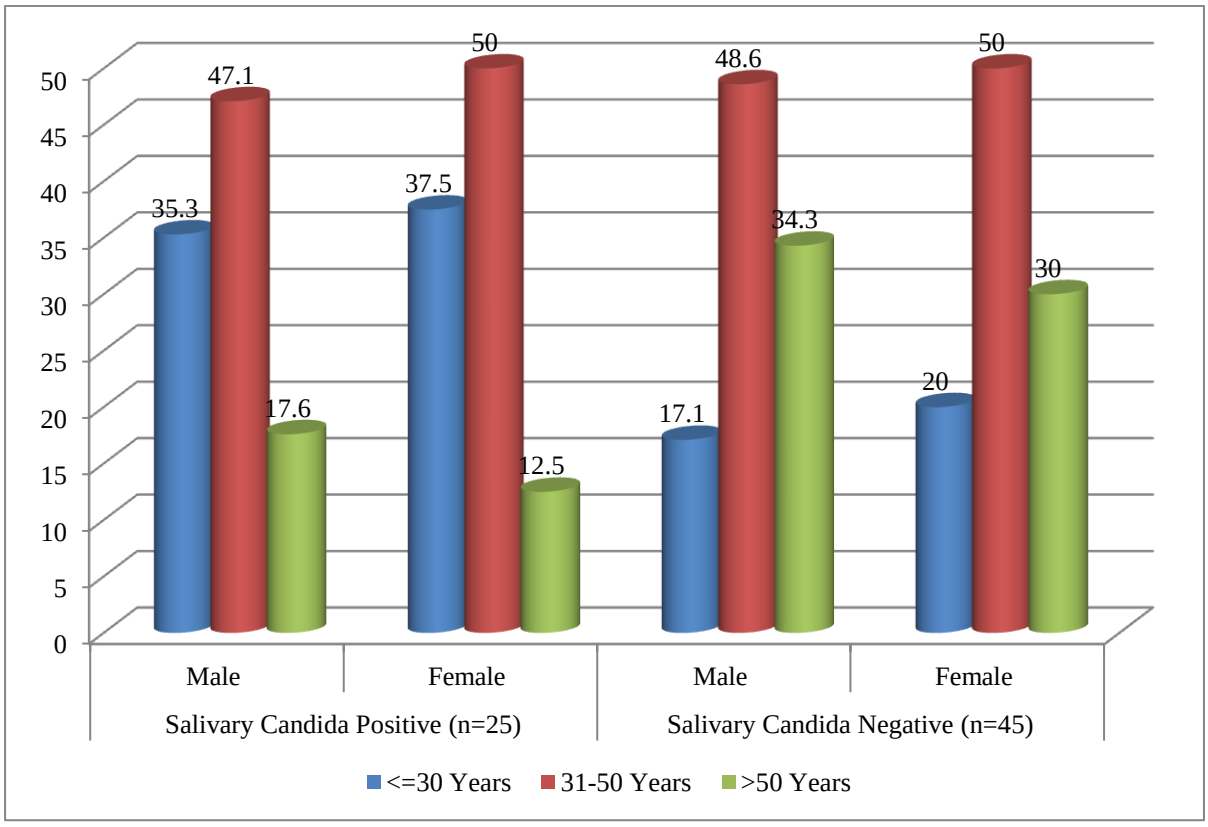


Graph 3: AGE AND GENDER DISTRIBUTION BETWEEN SALIVARY H. pylori POSITIVE AND NEGATIVE SUBJECTS N=70

AGE AND GENDER DISTRIBUTION BETWEEN SALIVARY *Candida* POSITIVE NEGATIVE SUBJECTS (n=70)

Table 4:

Age Groups	Salivary Candida Positive (n=25)						Salivary Candida Negative (n=45)					
	Male		Female		Total		Male		Female		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
<=30	6	35.3	3	37.5	9	36	6	17.1	2	20	8	17.8
31-50	8	47.1	4	50	12	48	17	48.6	5	50	22	48.9
>50	3	17.6	1	12.5	4	16	12	34.3	3	30	15	33.3
Total	17	68	8	32	25	100	35	77.8	10	22.2	45	100
p value	0.948						0.960					



Graph 4: AGE AND GENDER DISTRIBUTION BETWEEN SALIVARY *Candida* POSITIVE AND NEGATIVE SUBJECTS N=70

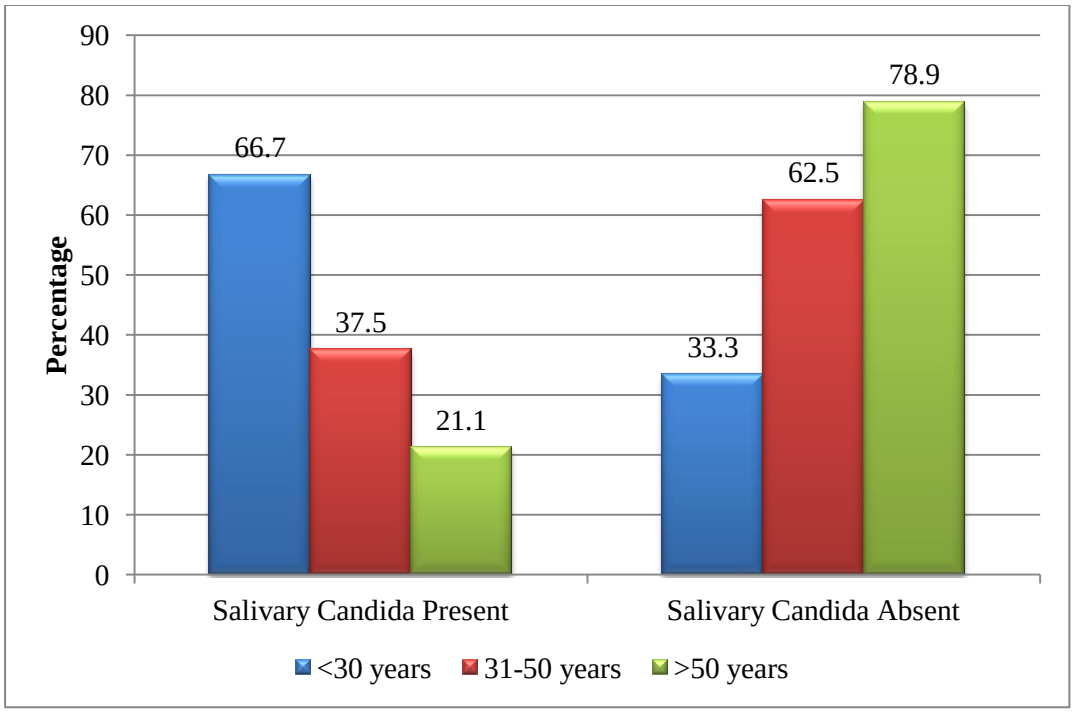
Table 5: RELATION BETWEEN AGE AND SALIVARY CANDIDAL STATUS IN SYMPTOMATIC GASTRITIS SUBJECTS (n=60)

Age Group	Candida in the Saliva		OR	95% CI	p-value^	p-value
	Present	Absent				
<30	6 (66.7%)	3 (33.3%)	7.5	0.98-68.75	0.035*	0.064
30-50 (R)	12 (37.5%)	20 (62.5%)	2.25	0.52-10.37	0.36	
>50	4 (21.1%)	15 (78.9%)				

Pearson chi-square between the age and salivary candidal status p=0.064

R- reference category

^- p value for odds ratio



Graph 5: RELATION BETWEEN AGE AND SALIVARY CANDIDAL STATUS IN SYMPTOMATIC SUBJECTS N=60

Table 6: CORRELATION COEFFICIENT BETWEEN VARIABLES:

S. No.	Variables	Co-relation coefficient	p value
1.	Age and Oral Candida (n=70)	r= - 0.296	0.022*
2.	Age and Oral Candida in Gastric H. pylori positive (n=30)	r= - 0.211	0.26
3.	Age and Oral Candida in Gastric H. pylori negative(n=30)	r= - 0.331	0.074
4.	Age and Oral Candida in Control (n=10)	r= - 0.327	0.356
5.	Gastric HP & Salivary HP (n=60)	r= 0.129	0.325
6	Salivary HP & Oral Candida (n=60)	r= 0.003	0.982
7.	Gastric HP & Oral Candida (n=60)	r= 0.208	0.112

ODD'S RATIO FOR GASTRITIS:

Table 7: ODD'S RATIO - SALIVARY *Helicobacter pylori* IN THE ORAL CAVITY:

SALIVARY H. pylori	SYMPTOMATIC GASTRITIS		OR	95% CI	P value
	H. pylori Positive (n=30)	H. pylori Negative (n=30)			
Positive	7 (23.3%)	4 (13.3%)	1.98	0.51-7.6	0.506
Negative (R)	23 (76.7%)	26 (86.7%)			
	30	30			

Table 8: ODD'S RATIO – ORAL CANDIDIASIS IN THE ORAL CAVITY:

ORAL CANDIDA	SYMPTOMATIC GASTRITIS		OR	95% CI	P value
	H. pylori Positive (n=30)	H. pylori Negative (n=30)			
Positive	14 (63.6%)	8 (36.4%)	2.4	0.82-7.1	0.506
Negative (R)	16 (42.1%)	22 (57.9%)			
	30	30			

Table 9: ODD'S RATIO – ORAL CANDIDIASIS AND SALIVARY *Helicobacter pylori* IN THE ORAL CAVITY:

ORAL CANDIDA AND HP	SYMPTOMATIC GASTRITIS		OR	95% CI	P value
	H. pylori Positive (n=30)	H. pylori Negative (n=30)			
Positive	4 (13.3%)	0	8.22	1.1-61.495	0.038*
Negative (R)	26 (86.7%)	30 (100%)			
	30	30			

Photographs

ARMAMENTARIUM:

Figure 1:

DNA EXTRACTION KIT

Figure 2:

PULSE VORTEX

Figure 3:

COOLING CENTRIFUGE

Figure 4:

COLLECTING TUBE AND GD COLUMN

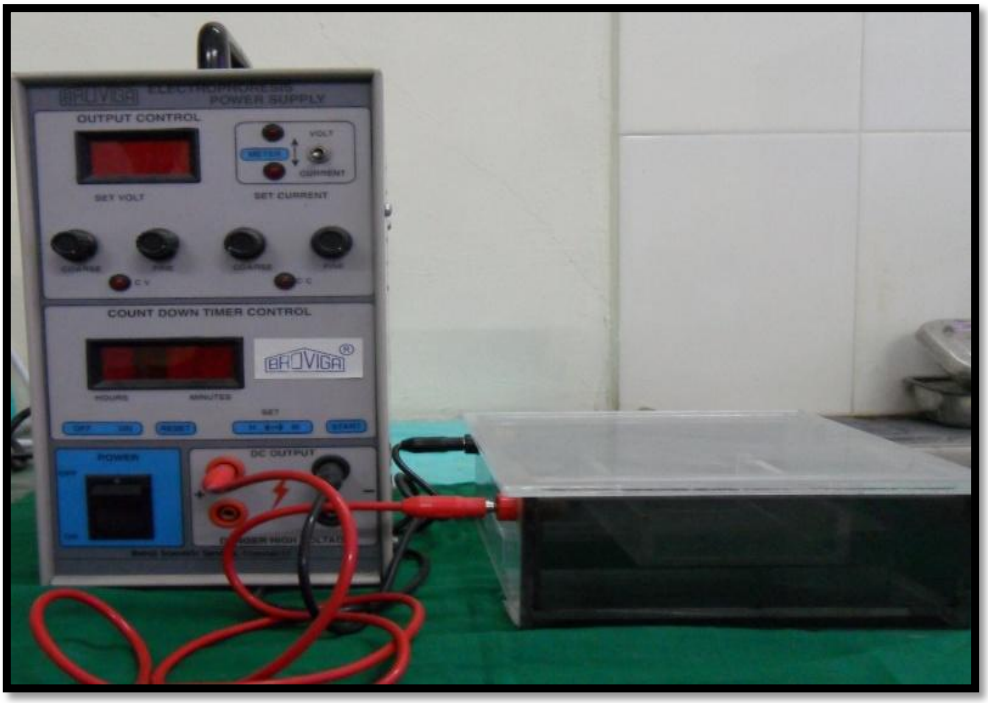
Figure 5:

MICROCENTRIFUGE TUBE

Figure 6:

AUTOMATED THERMAL CYCLER

Figure 7:

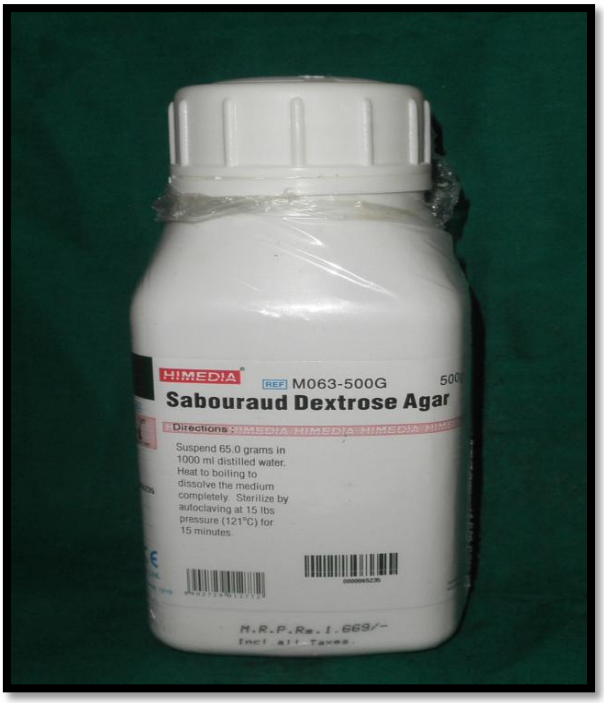


GEL ELECTROPHORESIS UNIT

Figure 8:

UV GEL DOCK SYSTEM

Figure 9:

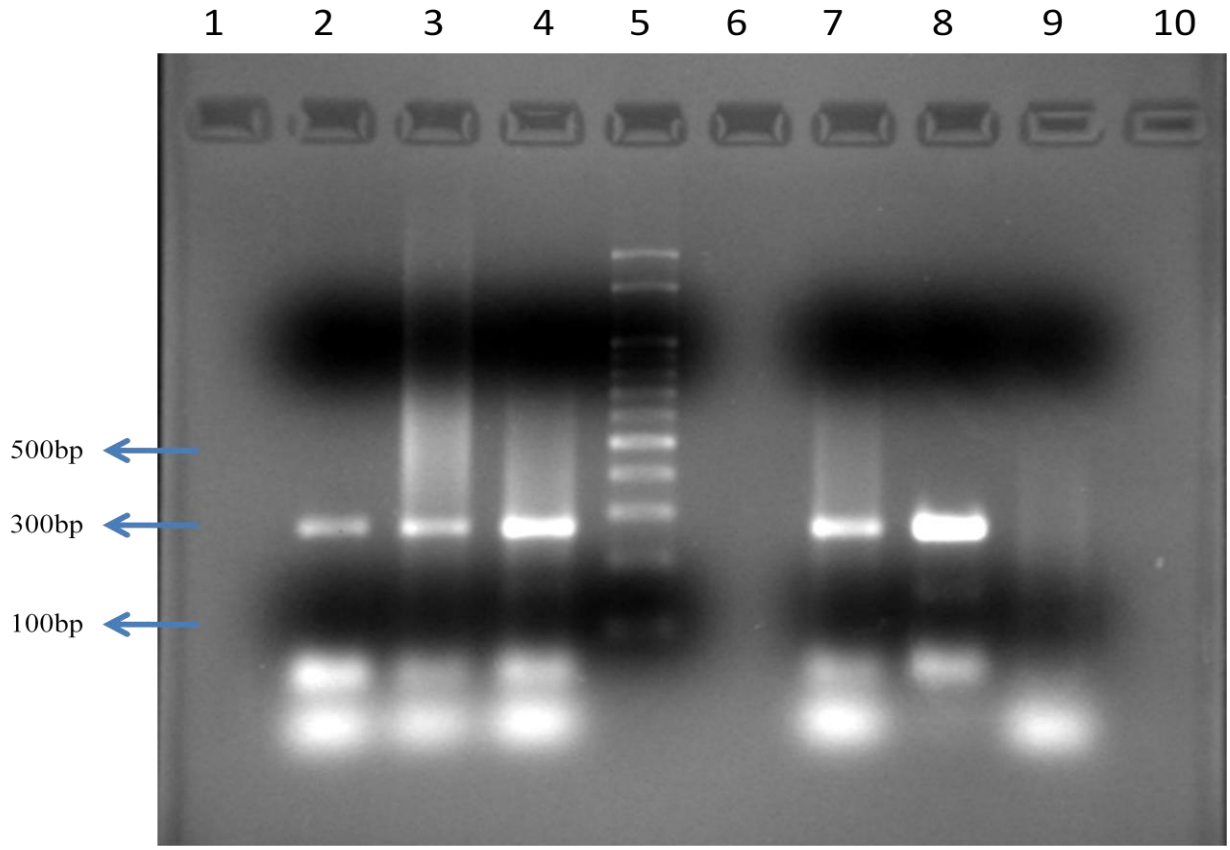


SABOURAUD DEXTROSE AGAR

Figure 10:

STERILE PETRIDISH

Figure 11:



Gel image showing band in the region of β-globulin (house keeping gene) corresponding to 268 base pairs where both primers for 16S rDNA and β-globulin were mixed together

Lane 1 : Empty

Lane 2 : Negative control

Lane 3 : Positive control

Lane 4 : Sample S-034 showing positive band for β-globulin in 268 bp region

Lane 5 : Empty

Lane 6 : 100 bp DNA ladder

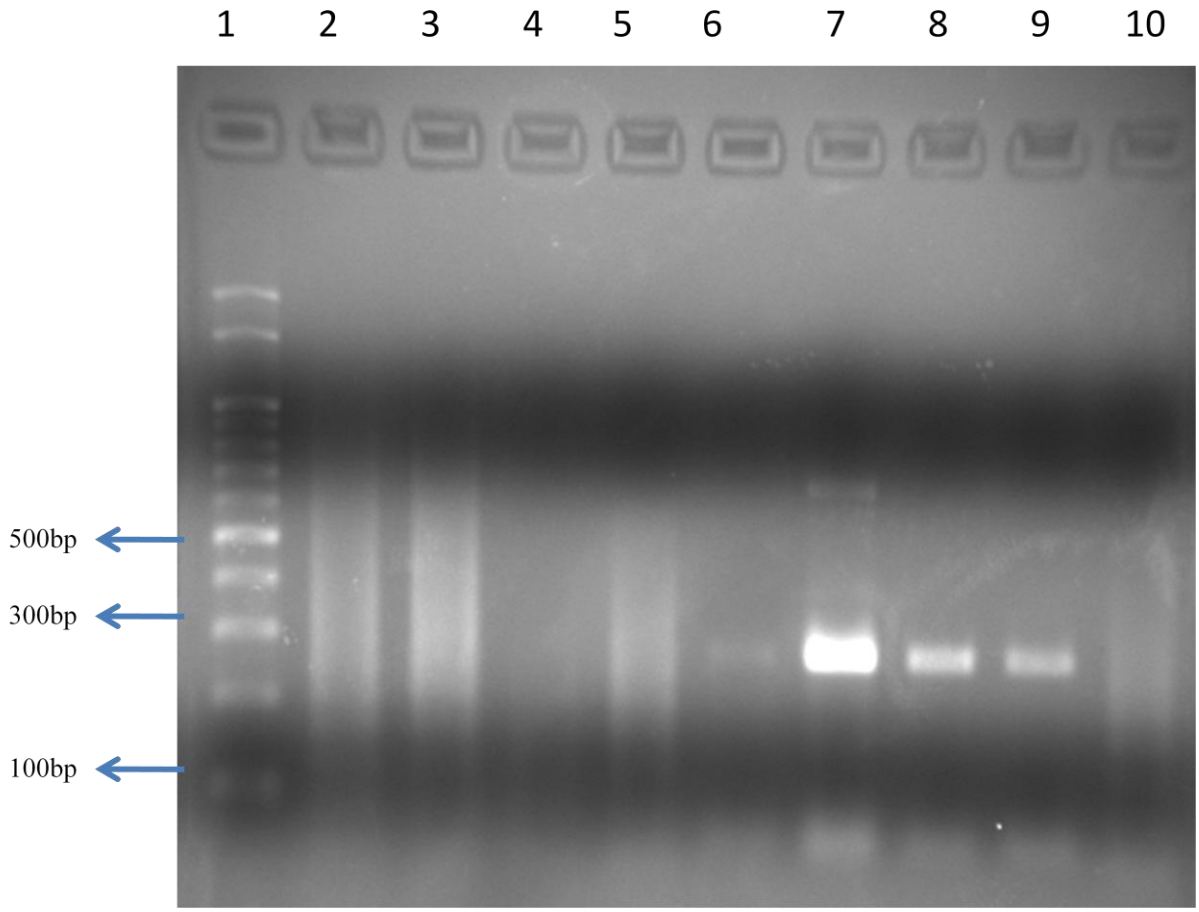
Lane 7 : Sample S-008 showing positive band for β-globulin in 268 bp region

Lane 8 :Sample S-001 showing positive band for β-globulin in 268 bp region

Lane 9 : Sample S-003 showing positive band for β-globulin in 268 bp region

Lane 10 : Empty

Figure 12:



Gel image showing band in the region of β-globulin (house keeping gene) corresponding to 268 base pairs where 16S rDNA and β-globulin were separately used.

Lane 1 : DNA Ladder

Lane 2 : Positive control showing no band for H. pylori DNA amplification (534bp)

Lane 3 : Sample S-050 showing no band for H. pylori DNA amplification (534bp)

Lane 4 : Sample S-109 showing no band for H. pylori DNA amplification (534bp)

Lane 5 : Sample S-086 showing no band for H. pylori DNA amplification (534bp)

Lane 6 : Sample S-086 showing mild band for β-globulin (268bp)

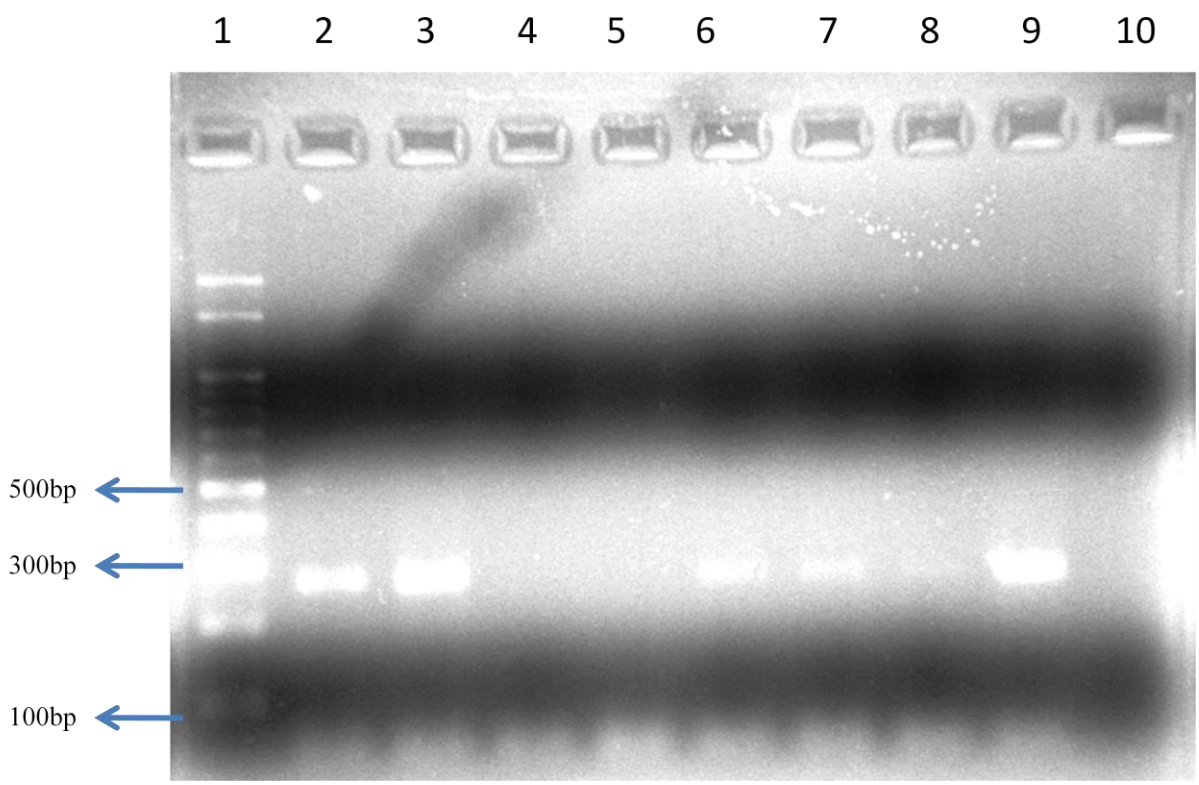
Lane 7 : Positive control showing positive band for β-globulin in 268 bp region

Lane 8 : Sample S-050 showing positive band for β-globulin in 268 bp region

Lane 9 : Sample S-109 positive band for β-globulin in 268 bp region

Lane 10 : Negative control

Figure 13:



Gel image showing band in the region of β-globulin (house keeping gene) corresponding to 268 base pairs where only β-globulin primers were added.

Lane 1 : DNA Ladder

Lane 2 : Positive control showing positive band for β-globulin in 268 bp region

Lane 3 : Sample S-055 showing positive band for β-globulin in 268 bp region

Lane 4 : Sample S-002 showing no band for β-globulin in 268 bp region

Lane 5 : Sample S-012 showing no band for β-globulin in 268 bp region)

Lane 6 : Sample S-089 showing mild band for β-globulin (268bp)

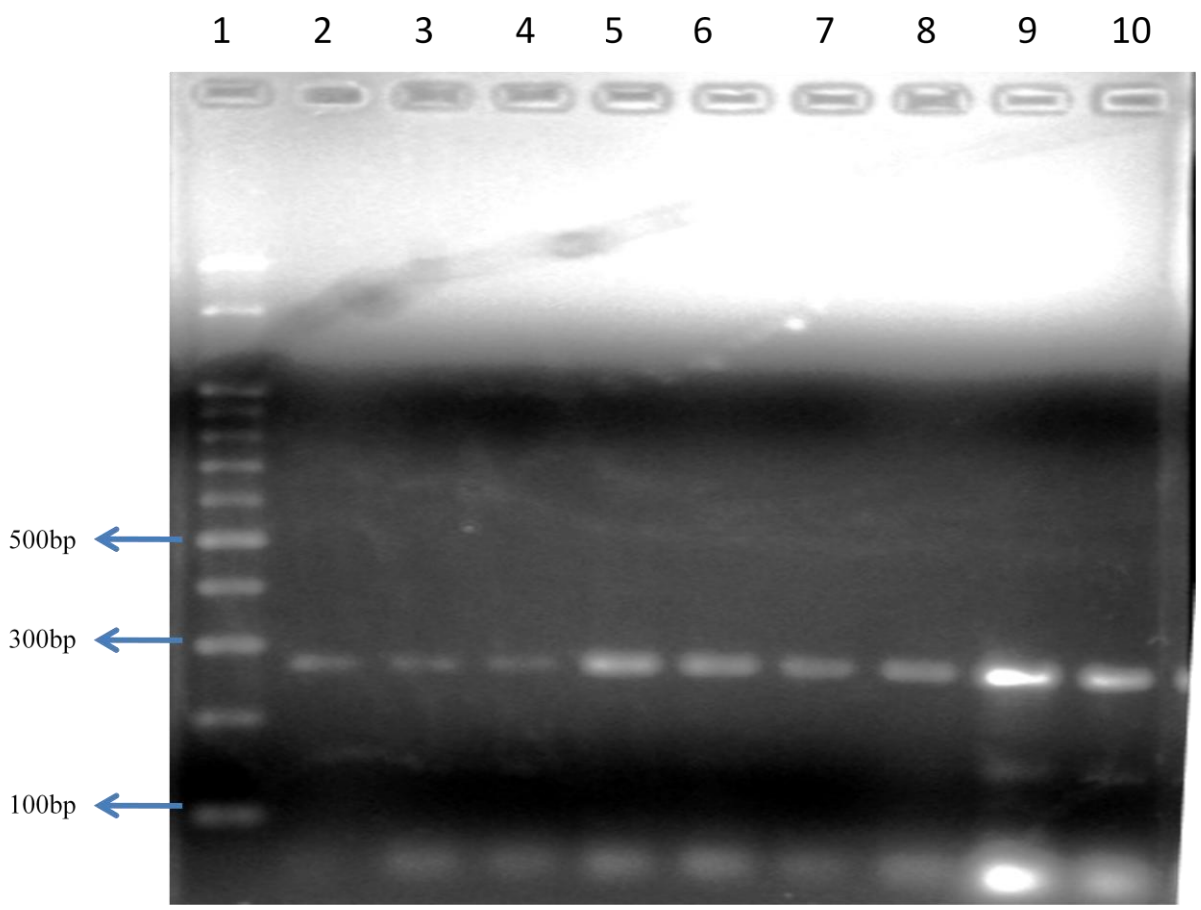
Lane 7 : Sample S-061 showing mild band for β-globulin (268bp)

Lane 8 : Sample S-120 showing mild band for β-globulin (268bp)

Lane 9 : Sample S-100 positive band for β-globulin in 268 bp region

Lane 10 : Negative control

Figure 14:



Gel image showing band in the region of β-globulin (house keeping gene) corresponding to 268 base pairs with only β-globulin amplification

Lane 1 : DNA Ladder

Lane 2 : Sample S-114 showing positive band for β-globulin in 268 bp region

Lane 3 : Sample S-030showing positive band for β-globulin in 268 bp region

Lane 4 : Sample S-036 showing positive band for β-globulin in 268 bp region

Lane 5 : Positive control showing band for β-globulin in 268 bp region)

Lane 6 : Sample S-097 showing band for β-globulin (268bp)

Lane 7 : Sample S-098 showing band for β-globulin (268bp)

Lane 8 : Sample S-122 showing band for β-globulin (268bp)

Lane 9 : Sample S-124 positive band for β-globulin in 268 bp region

Lane 10 : Sample S-070 positive band for β-globulin in 268 bp region

DETECTION OF HELICOBACTER PYLORI BY REAL TIME POLYMERASE CHAIN REACTION

Figure 15:

Pos	Name	Ct FAM	Amount FAM [Copies]	Target FAM
B1	UKN-S001		-	HP
B2	UKN-S003		-	HP
B3	UKN-S034		-	HP
B4	UKN-S045		-	HP
B5	UKN-S058		-	HP
B6	UKN-S061		-	HP
B7	UKN-S071		-	HP
B8	UKN-S074		-	HP
B9	UKN-S079		-	HP
B10	UKN-S080		-	HP
B11	UKN-S086		-	HP
B12	UKN-S089		-	HP
C1	UKN-S090		-	HP
C2	UKN-S092		-	HP
C3	UKN-S093		-	HP
C4	UKN-S097		-	HP
C5	UKN-S098		-	HP
C6	UKN-S110		-	HP
C7	UKN-S112		-	HP
C8	UKN-S120		-	HP
C9	UKN-S121		-	HP
C10	UKN-S122		-	HP
C11	UKN-S016	35.05	-	HP
C12	UKN-S124		-	HP
D1	UKN-S002		-	HP
D2	UKN-S004		-	HP
D3	UKN-S007		-	HP
D4	UKN-S008		-	HP
D5	UKN-S021		-	HP
D6	UKN-S026		-	HP
D7	UKN-S028		-	HP
D9	UNK-S029		-	HP
E1	UKN-S030		-	HP
E2	UKN-N001		-	HP
E3	UKN-N002		-	HP
E4	UKN-N003		-	HP

TABLE DEPICTING THE CYCLE OF AMPLIFICATION INITIATION

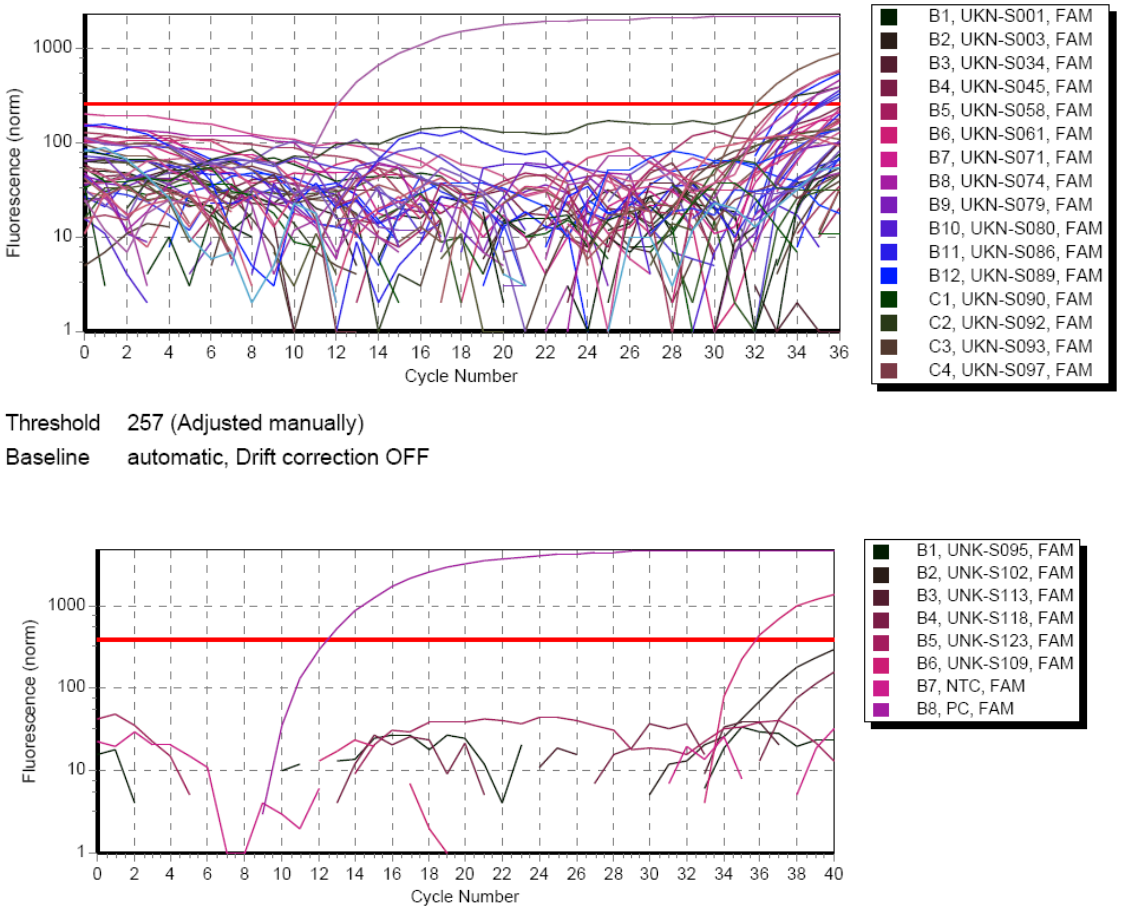
Figure 16:

Pos	Name	Ct FAM	Amount FAM [Copies]	Target FAM
E5	UKN-N004		-	HP
E6	UKN-N005		-	HP
E7	UKN-N006		-	HP
E8	UKN-S037	34.51	-	HP
E9	UKN-N007		-	HP
E10	UKN-S050	34.92	-	HP
E11	UKN-N008		-	HP
E12	UKN-S055	33.61	-	HP
F1	UKN-N009		-	HP
F2	UKN-S067	32.82	-	HP
F3	UKN-N010		-	HP
F4	UKN-S036		-	HP
F5	UKN-S100	33.09	-	HP
F6	UKN-S039		-	HP
F7	UNK-S042		-	HP
F8	UKN-S114	34.07	-	HP
F9	UKN-S043		-	HP
F10	UKN-S019	35.27	-	HP
F11	UKN-S049		-	HP
F12	UKN-S053		-	HP
G1	UKN-S062		-	HP
G2	UKN-066		-	HP
G3	UKN-S070		-	HP
G4	UKN-2S057	31.97	-	HP
G5	UKN-S078		-	HP
G6	UKN-S083		-	HP
G7	UKN-S094	33.28	-	HP
G8	PC	12.03	-	HP
G9	NTC	-	-	HP
G10	NTC	-	-	HP

Pos	Name	Ct FAM	Amount FAM [Copies]	Target FAM
B1	UNK-S095		-	HP
B2	UNK-S102		-	HP
B3	UNK-S113		-	HP
B4	UNK-S118		-	HP
B5	UNK-S123		-	HP
B6	UNK-S109	35.80	-	HP
B7	NTC	-	-	HP
B8	PC	12.47	-	HP

TABLE DEPICTING THE CYCLE OF AMPLIFICATION INITIATION

Figure 17:



Amplification plot showing the positive samples crossing the threshold fam.

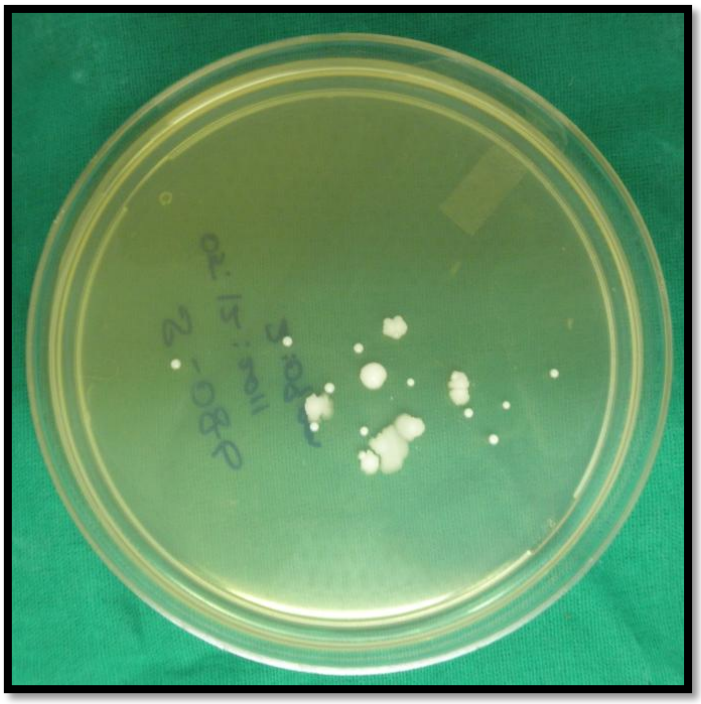
Samples crossin teh Threshold FAM are considered positive for *Helicobacter pylori*

DETECTION OF CANDIDA BY SABOURAUD'S DEXTROSE AGAR:

Figure 18:

Candidal colonies formed in Sabouraud Dextrose Agar medium after 48 hours incubation at 37 ^{o} C

Figure 19:



Candidal colonies formed in SDAgar medium after 48 hours incubation at 37 ^{o} C

Discussion

The significance of *Helicobacter pylori* in the field of gastroenterology was first documented by Marshall and Warren in 1983. Subsequently, *Helicobacter pylori's* role has been defined in the development and recurrence of gastric and duodenal ulcers. The World Health Organisation has now designated *Helicobacter pylori* as type I carcinogen because of its association with gastric adenocarcinoma and it has also been related with other clinical entities such as Mucosa Associated Lymphoid Tissue lymphoma.[51] The presence of *Helicobacter pylori* in the oral cavity has been reported by various investigators, who also postulated that it could be the source for stomach infection/gastritis and re-infection after eradication therapy and it could have an active role in the person to person to transmission.[10,52,53]

Dowsett et al[12] have also confirmed that given the fact that the reservoir for *Helicobacter pylori* has not yet been confirmed, the theory of person to person spread is now generally accepted even though it needs to be proven. However, the route of infection remains to open conjuncture. The oral cavity supports many ecological niches, some of which may provide the micro-aerophilic environment necessary for *Helicobacter pylori* survival and its multiplication.

Several studies have detected *Helicobacter pylori* in the human oral cavity and some have also suggested that oral cavity is the primary extragastric reservoir.[10,53,54] In contrast, other studies have failed to find evidence supporting the role of the oral cavity as the major reservoir.[4,55] Some investigators [56] believed that *Helicobacter pylori* belongs to the normal flora of the human oral cavity and that it maintains a commensal relationship with the human host. [57,58] However, *Helicobacter pylori* colonizes the oral cavity intermittently as a result of

ingestion of contaminated food or due to gastroesophageal reflux. These observations raise a hypothesis as to whether oral cavity could be the reservoir of the *Helicobacter pylori* for gastric re-infection. Mapstone et al [59] have described in their work that the culturable forms of *Helicobacter pylori* have not been recovered from water sources or food and therefore it is not clear as to how *Helicobacter pylori* gets the access to the oral cavity. Ali Hatef Salmanian et al [25] in their study of association of yeast of the oral cavity as a reservoir of *Helicobacter pylori* stated that the yeast, *Candida* may facilitate the colonization of *Helicobacter pylori* inside the human oral cavity. They have also detailed that the microscopic observation of their wet mount preparation revealed the existence of bacterium like bodies inside the yeast vacuoles.

Madinier et al [60] stated that patients with *Helicobacter pylori* isolated from oral specimens, have *Helicobacter pylori* detected even on the gastric biopsy, but many patients with gastric *Helicobacter pylori* do not exhibit oral co-infection. Sillness et al [61] stated that the correlation of *Helicobacter pylori* in oral specimens and gastric biopsies are very inconsistent and not in agreement with the world wide prevalence *Helicobacter pylori* in the stomach. Burgers et al [62] have stated that the detection of *Helicobacter pylori* in the mouth can occur independently of stomach colonization, suggesting that the human oral cavity could represent an important extra gastric reservoir for *Helicobacter pylori.* [8,63]

The current focus of interest concerning *Helicobacter pylori* is its association with yeast in the oral cavity because these two microorganisms occur in both the oral cavity and in certain areas of gastrointestinal system of the human.

Even though, the gold standard for detection of *Helicobacter pylori* is Giemsa stained histological detection, other methods such as rapid urease test, culture and non invasive methods such as serologic and urea breath test are used to diagnose or monitor presence of *Helicobacter pylori*. [64] Sometimes, low number of *Helicobacter pylori* cells are isolated from the gastric environment due to difficulty in culturing *Helicobacter pylori* from sites colonized by fast-growing bacteria and the presence of bacteria entering the viable but not culturable (VBNC) coccoid . [65, 66] So, for better sensitivity in *Helicobacter pylori* detection molecular methods such as polymerase chain reaction assays are used to detect the bacterium in samples such as gastric secretions, saliva, dental plaque and stool. [22,63,67-73]

Our study was designed to evaluate the presence of *Helicobacter pylori* in the oral cavity of patients who presented with symptomatic gastritis. The study group was further characterized as those who were *Helicobacter pylori* positive in the gastric mucosa, *Helicobacter pylori* negative in the gastric mucosa and a control group.

To detect the presence of *Helicobacter pylori* in the saliva, we extracted DNA from the saliva samples. The extracted DNA was subjected to conventional polymerase chain reaction (PCR) using primers for 16S rDNA genome. Beta globulin primers PCO4 and GH 20 were selected to amplify the β chain of haemoglobin to the validate the PCR amplification. Positive sample from a previous study to detect *Helicobacter pylori* was used as our positive control. Millipore water was used instead of the sample in master mix preparation as negative control.

The amplified products were subjected to gel electrophoresis, to detect the presence of *Helicobacter pylori* and beta globulin to validate the PCR amplification. The gel electrophoresis result yielded a band in the region of 268 base pairs corresponding to the beta globulin. But, we were unable to obtain a positive band in the region of 534 base pair corresponding to our target 16S rDNA genome for *helicobacter pylori* even in our positive control.

We modified our technique with different denaturation, annealing and extension temperatures and varied their duration in an attempt to detect *Helicobacter pylori*. Our repeated amplification at various temperatures and durations did not show the band corresponding to *Helicobacter pylori*, in our positive controls. Given this situation, where the amplification of the house keeping gene was seen in the modified methodologies, we substantiated that our technique was accurate. Subsequently, we opted for the detection of *Helicobacter pylori* by real time PCR because this technique is more sensitive. This was successful and yielded reproducible results.

From our study results, we found that among the overall study population about 48 % were in the 3rd to 5th decade of life. And the overall male: female ratio was 3:1. In the individual group, we found that the male: female ratio in patients with symptomatic gastritis was 4:1 and this similarity was seen in both symptomatic gastritis patients with *Helicobacter pylori* positive in the gastric mucosa and symptomatic gastritis patients with *Helicobacter pylori* negative in the gastric mucosa. Alejandra Berroteran et al [10] found a higher prevalence of *Helicobacter pylori* in their study in the gastric samples from females (62.5%) than in the males (37.5%). Similar seroprevalences of *Helicobacter pylori* was

distributed in the stomach samples of males and females as observed by Gasbarrini G et al. [74] The gender distribution in our study is in concurrence with the findings of Goodman KJ et al and Fawcett JP et al.[75,76] Among the subjects with the presence of *Helicobacter pylori* in the saliva (n=11), we found that 81.8% were males and in the subjects without *Helicobacter pylori* in the saliva (n=59), 72.9% were males. Similarly, we found a male predominance with respect to detection of *Candida* in the saliva.

We detected *Helicobacter pylori* in the saliva of 7 patients who were *Helicobacter pylori* positive in the gastric mucosa (Group I) and 4 patients who were *Helicobacter pylori* negative in the gastric mucosa (Group II). We studied the correlation between the presence of *Helicobacter pylori* in the saliva of symptomatic gastritis patients and gastric in the gastric mucosa , but we did not find a significant correlation.

We isolated candida in 22 patients in our study group of which 46.6% were in the group I and 26.6% were group II. We did not find any correlation between the presence of candida in the saliva and presence of *Helicobacter pylori* in gastric mucosa.

In our study, we observed that symptomatic gastritis patients who were less than 30 years of age had 7.5 (O.R) times (p=0.035*) more chance of having oral *Candida* than those who were above 50 years of age group and we also found that this association was statistically significant (p=0.064). With respect to oral candidal carriage, there was no difference in the mean CFU/ml in the overall study group. We observed that as the age increases the mean colony forming units

(CFU/ml) also increased in the overall study group. This finding could be due to the fact that older individuals have other local and systemic factors which could influence and provide a suitable environment for the growth of *Candida*.[26] There was no significant difference in the mean CFU between the groups. Though we did not find a significant difference in the mean CFU/ml between the age groups, we observed that the mean CFU/ml increased with age in patients with symptomatic gastritis in both males and females, when stratified.

When we studied the correlation between patients who had both candida and *Helicobacter pylori* in their saliva, we found that patients who had both these organisms detected in the saliva, had 8 times higher chance of having gastritis with *Helicobacter pylori* being positive in the gastric mucosa and this correlation was statistically significant (p=0.038*).

The presence of *Helicobacter pylori* and *Candida* in saliva of the symptomatic gastritis patients supports the hypothesis that *Helicobacter pylori* can be harboured within the *Candida* as proposed by some investigators. [25]

The findings of this preliminary study could be further explored to determine if the oral cavity is the reservoir for *Helicobacter pylori*.

Summary & Conclusion

In our group comprising of 70 subjects, 60 patients presented with symptomatic gastritis and 10 were asymptomatic subjects who served as the control group. The symptomatic gastritis subjects had undergone endoscopy and gastric biopsy specimen was collected. Histopathologically, the biopsy specimen was observed for the presence of *Helicobacter pylori*. The subjects were divided into 3 groups.

Group I comprised of 30 subjects with *Helicobacter pylori* in their gastric mucosa.
Group II comprised of 30 subjects without *Helicobacter pylori* in their gastric mucosa.
Group III comprised of 10 asymptomatic controls.

- We found that there was a male predominance for gastritis in our study groups. The symptomatic gastritis was more commonly seen between the 3^{rd} and 5^{th} decade of life.
- Our study findings conclude that subjects who are less than 30 years of age are more prone to have *Candida* in their saliva.
- There was no difference in the mean CFU between the study groups and the mean CFU between the age groups did not show statistical difference. But, when stratified between gender, there was an increase in CFU with increasing age.
- We found that oral *candida* carriage was more frequently seen in the younger age group and odd's have shown that when compared to subjects above 50 years, symptomatic subjects less than 30 years have 7.5 times higher risk of getting salivary candidal carriage.

- We found that there is no correlation between the presence or absence of salivary *Helicobacter pylori* and the presence or absence of *Helicobacter pylori* in the gastric mucosa. There was no correlation between salivary *candida* and the presence or absence of Helicobacter pylori in the gastric mucosa.
- In our study, the patients with *Candida* and *Helicobacter pylori* in the saliva had 8 times more chance of having gastritis with Helicobacter pylori positivity in their gastric mucosa than in whom these organisms were not detected.
- Further studies are needed to understand the relationship of *Helicobacter pylori*, *Candida* and gastritis.

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Annexure

ANNEXURE 1:

From,

Institutional Review Board,

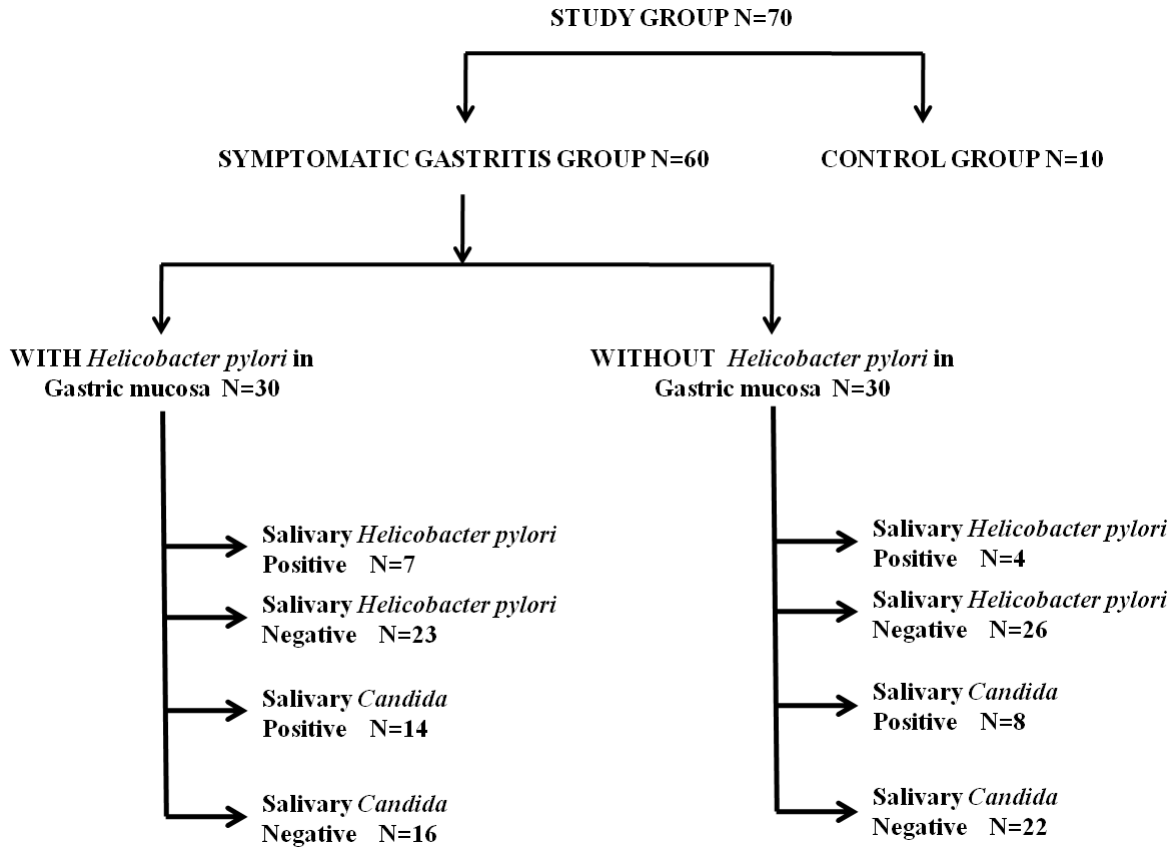
Ragas Dental College and Hospital,

Uthandi,Chennai

The dissertation topic titled '**Evaluation of coexistence of *Helicobacter pylori* and *Candida* in the oral cavity of dyspeptic patients**' submitted by **Dr. Prem Karthick B** has been approved by the Institutional Review Board of Ragas Dental College and Hospital on 14th March 2011.

Dr. K. Ranganathan	**Dr. S. Ramachandran**
Secretary,	**Chairman,**
Ragas, IRB	**Ragas, IRB**

ANNEXURE 2:


STUDY GROUP N=70
SYMPTOMATIC GASTRITIS GROUP N=60
CONTROL GROUP N=10
WITH Helicobacter pylori in Gastric mucosa N=30
WITHOUT Helicobacter pylori in Gastric mucosa N=30
Salivary Helicobacter pylori Positive N=7
Salivary Helicobacter pylori Negative N=23
Salivary Candida Positive N=14
Salivary Candida Negative N=16
Salivary Helicobacter pylori Positive N=4
Salivary Helicobacter pylori Negative N=26
Salivary Candida Positive N=8
Salivary Candida Negative N=22